SUPPLEMENT I
TO
THE JAPANESE PHARMACOPOEIA
EIGHTEENTH EDITION

Official from December 12, 2022

English Version

THE MINISTRY OF HEALTH, LABOUR AND WELFARE

Notice: This *English Version* of the Japanese Pharmacopoeia is published for the convenience of users unfamiliar with the Japanese language. When and if any discrepancy arises between the Japanese original and its English translation, the former is authentic.

Printed in Japan

The Ministry of Health, Labour and Welfare Ministerial Notification No. 355

Pursuant to Paragraph 1, Article 41 of Act on Securing Quality, Efficacy and Safety of Products Including Pharmaceuticals and Medical Devices (Law No. 145, 1960), we hereby revise a part of the Japanese Pharmacopoeia (Ministerial Notification No. 220, 2021) as follows*.

KATO Katsunobu
The Minister of Health, Labour and Welfare

December 12, 2022

Japanese Pharmacopoeia

(The text referred to by the term ''as follows'' are omitted here. All of the revised Japanese Pharmacopoeia in accordance with this notification are made available for public exhibition at the Pharmaceutical Evaluation Division, Pharmaceutical Safety and Environmental Health Bureau, Ministry of Health, Labour and Welfare, at each Regional Bureau of Health and Welfare, and at each Prefectural Office in Japan, and made public by publishing it on the website of the Ministry of Health, Labour and Welfare.)

Supplementary Provisions

(Effective Date)

Article 1 This Notification is applied from the date of the notification. (referred to as the ''notification date'' in the next and third articles)

(Transitional measures)

Article 2 In the case of drugs which are listed in the Japanese Pharmacopoeia (hereinafter referred to as ''previous Pharmacopoeia'') [limited to those listed in the Japanese Pharmacopoeia whose standards are changed in accordance with this notification (hereinafter referred to as ''new Pharmacopoeia'')] and drugs which have been approved as of the notification date as prescribed under Paragraph 1, Article 14 of the same law [including drugs the Minister of Health, Labour and Welfare specifies (the Ministry of Health and Welfare Ministerial Notification No. 104, 1994) as of the day before the notification date as those exempted from marketing approval pursuant to Paragraph 1, Article 14 of the same law (hereinafter referred to as ''drugs exempted from approval'')], the Name and Standards established in the previous Pharmacopoeia (limited to part of the Name and Standards for the drugs concerned) may be accepted to conform to the Name and Standards established in the new Pharmacopoeia before and on June 30, 2024.

Article 3 In the case of drugs which are listed in the new Pharmacopoeia (excluding those listed in the previous Pharmacopoeia) and drugs which have been approved as of the notification date as prescribed under the Paragraph 1, Article 14 of the same law (including those exempted from approval), they may be accepted as those being not listed in the new Pharmacopoeia before and on June 30, 2024.

*The term ''as follows'' here indicates the content of Supplement I to the Japanese Pharmacopoeia Eighteenth Edition from General Tests, Processes and Apparatus to Ultraviolet-visible Reference Spectra (pp. 2807 – 2930).

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PREFACE

The 18th Edition of the Japanese Pharmacopoeia (JP) was promulgated by Ministerial Notification No. 220 of the Ministry of Health, Labour and Welfare (MHLW) on June 7, 2021.

In July 2021, the Committee on JP established the basic principles for the preparation of the JP 19th Edition, setting out the roles and characteristics of the JP, the definite measures for the revision, and the date of the revision.

It was agreed that the JP should be an official document that defines the specifications, criteria and standard test methods necessary to properly assure the quality of medicines in Japan in response to the progress of science and technology and medical demands, in order to contribute to ensuring public health. It should define the standards for specifications, as well as the methods of testing to assure overall quality of all drugs in principle, and it should have a role in clarifying the criteria for quality assurance of drugs that are recognized to be essential for medical treatment. The JP has been prepared with the aid of the knowledge and experience of many professionals in the pharmaceutical field. Therefore, the JP should have the characteristics of an official standard, which might be widely used by all parties concerned, and it should play an appropriate role of providing information and understanding about the quality of drugs to the public. Moreover, as a pharmaceutical quality standard in the international community, it should play an appropriate role and contribute to the utilization of advanced technology and the promotion of international consistency in order to ensure the quality of drugs beyond the national level.

As the policy of the JP, the five basic principles, which we refer to as the ''five pillars'', were established as follows: 1) Enhancing listed articles by prioritizing drugs which are important from the viewpoint of health care and medical treatment; 2) Making qualitative improvement by introducing the latest science and technology; 3) Further promoting internationalization in response to globalization of drug market; 4) Making prompt partial revision as necessary and facilitating smooth administrative operation; and 5) Ensuring transparency regarding the revision, and disseminating the JP to the public domestically and internationally. It was agreed that the Committee on JP should make efforts, on the basis of these principles, to ensure that the JP is used more effectively in the fields of health care and medical treatment by taking appropriate measurements, including getting the understanding and cooperation of other parties concerned.

It was also agreed that JP articles should cover drugs, which are important from the viewpoint of health care and medical treatment, clinical performance or merits and frequency of use, as soon as possible after they reach the market.

The target date for the publication of JP 19th Edition (the Japanese edition) was set as April 2026.

JP drafts are discussed in the following committees that were established in the Pharmaceuticals and Medical Devices Agency: Expert Committee; Sub-committee on Manufacturing Process-related Matters; Committee on Chemicals; Committee on Antibiotics; Committee on Biologicals; Committee on Crude Drugs; Committee on Pharmaceutical Excipients; Committee on Physico-Chemical Methods; Committee on Drug Formulation; Committee on Physical Methods; Committee on Biological Methods; Committee on Nomenclature for Pharmaceuticals; Committee on International Harmonization; and Committee on Reference Standards. Furthermore, working groups are established under the Expert Committee, Committee on Biologicals, Committee on Pharmaceutical Excipients and Committee on Drug Formulation.

The committees initiated deliberations on the several revision. Draft revisions covering subjects in General Tests and Monographs, Ultraviolet-visible Reference Spectra, Infrared Reference Spectra, for which discussions were finished between September 2020 and June 2022, were prepared for a supplement to the JP 18.

Numbers of discussions in the committees to prepare the supplement drafts were as follows: Expert Committee (17, including working group); Sub-committee on Manufacturing Process-related Matters (1); Committee on Chemicals (23); Committee on Antibiotics (4); Committee on Biologicals (7); Committee on Crude Drugs (16); Committee on Pharmaceutical Excipients (13, including working group); Committee on Physico-Chemical Methods (8); Committee on Drug Formulation (17, including working group); Committee on Physical Methods (6); Committee on Biological Methods (6); Committee on Nomenclature for Pharmaceuticals (4); Committee on International Harmonization (7); and Committee on Reference

Standards (3, including working group).

It should be noted that in the preparation of the drafts for the supplement, generous cooperation was given by the Pharmaceutical Technology Committee of the Kansai Pharmaceutical Manufacturers Association, the Pharmacopeia and CMC Committee of the Pharmaceutical Manufacturers' Association of Tokyo, the Tokyo Crude Drugs Association, the International Pharmaceutical Excipients Council Japan, the Home Medicine Association of Japan, the Japan Kampo Medicines Manufacturers Association, the Japan Flavor and Fragrance Materials Association, the Japan Natural Medicines Association, the Japan Pharmaceutical Manufacturers Association, the Federation of Pharmaceutical Manufacturers' Association of Japan, the Parenteral Drug Association Japan Chapter, the Japan Reagent Association, the Japan Oilseed Processors Association, the Japan Analytical Instruments Manufacturers' Association, and the Asian Society of Innovative Packaging Technology.

The draft revisions were examined by the Committee on JP in July 2022, followed by the Pharmaceutical Affairs and Food Sanitation Council (PAFSC) in September 2022, and then submitted to the Minister of Health, Labour and Welfare. In the committee on JP, Mitsuru Hashida took the role of the chairman from January 2011 to December 2020, and Shigeru Ohta from January 2021 to December 2022.

In consequence of this revision, the Supplement I to the JP 18th Edition carries 2042 articles, owing to the addition of 11 articles and the deletion of 2 articles.

The principles of description and the salient points of the revision in this volume are as follows:

1. The Supplement I to the JP 18th Edition comprises the following items, in order: Notification of MHLW; Contents; Preface; General Tests, Processes and Apparatus; Official Monographs; then followed by Infrared Reference Spectra and Ultraviolet-visible Reference Spectra; General Information; and; as an appendix a Cumulative Index containing references to the main volume and Supplement I.

2. The articles in Official Monographs, Infrared Reference Spectra and Ultraviolet-visible Reference Spectra are respectively placed in alphabetical order in principle.

3. The following items in each monograph are put in the order shown below, except that unnecessary items are omitted depending on the nature of the drug:

(1) English title
(2) Commonly used name(s)
(3) Latin title (only for crude drugs)
(4) Title in Japanese
(5) Structural formula or empirical formula
(6) Molecular formula and molecular mass
(7) Chemical name
(8) Chemical Abstracts Service (CAS) Registry Number
(9) Origin
(10) Limits of the content of the ingredient(s) and/or the unit of potency
(11) Labeling requirements
(12) Method of preparation
(13) Manufacture
(14) Description
(15) Identification tests
(16) Specific physical and/or chemical values
(17) Purity tests
(18) Potential adulteration
(19) Loss on drying or Ignition, or Water
(20) Residue on ignition, Total ash or Acid-insoluble ash
(21) Tests being required for pharmaceutical preparations
(22) Other special tests
(23) Assay
(24) Containers and storage
(25) Shelf life
(26) Others

4. In each monograph, the following physical and chemical values representing the properties and quality of the drug are given in the order indicated below, except that unnecessary items are omitted depending on the nature of drug:

(1) Alcohol number
(2) Absorbance
(3) Congealing point
(4) Refractive index
(5) Osmolar ratio
(6) Optical rotation
(7) Constituent amino acids
(8) Viscosity
(9) pH
(10) Content ratio of the active ingredients
(11) Specific gravity
(12) Boiling point
(13) Melting point
(14) Acid value
(15) Saponification value
(16) Ester value
(17) Hydroxyl value
(18) Iodine value

5. Identification tests comprise the following items, which are generally put in the order given below:
(1) Coloration reactions
(2) Precipitation reactions
(3) Decomposition reactions
(4) Derivatives
(5) Infrared and/or ultraviolet-visible absorption spectrometry
(6) Nuclear magnetic resonance spectrometry
(7) Chromatography
(8) Special reactions
(9) Cations
(10) Anions

6. Purity tests comprise the following items, which are generally put in the order given below, except that unnecessary items are omitted depending on the nature of drug:
(1) Color
(2) Odor
(3) Clarity and/or color of solution
(4) Acidity or alkalinity
(5) Acidity
(6) Acidity Alkalinity
(7) Chloride
(8) Sulfate
(9) Sulfite
(10) Nitrate
(11) Nitrite
(12) Carbonate
(13) Bromide
(14) Iodide
(15) Soluble halide
(16) Thiocyanate
(17) Selenium
(18) Cationic salts
(19) Ammonium
(20) Heavy metals
(21) Iron
(22) Manganese
(23) Chromium
(24) Bismuth
(25) Tin
(26) Aluminum
(27) Zinc
(28) Cadmium
(29) Mercury
(30) Copper
(31) Lead
(32) Silver
(33) Alkaline earth metals
(34) Arsenic
(35) Free phosphoric acid
(36) Foreign matters
(37) Related substances
(38) Isomer
(39) Enantiomer
(40) Diastereomer
(41) Polymer
(42) Residual solvent
(43) Other impurities
(44) Residue on evaporation
(45) Readily carbonizable substances

7. The following item was newly added to General Tests, Processes and Apparatus:
(1) 2.00 Chromatography
(2) 2.27 Near Infrared Spectrometry
(3) 2.28 Circular Dichroism Spectroscopy

8. The following items in General Tests, Processes and Apparatus were revised:
(1) 2.01 Liquid Chromatography
(2) 2.02 Gas Chromatography
(3) 2.22 Fluorometry
(4) 2.58 X-Ray Powder Diffraction Method
(5) 3.04 Particle Size Determination
(6) 9.01 Reference Standards
(7) 9.41 Reagents, Test Solutions
(8) 9.42 Solid Supports/Column Packings for Chromatography

9. The following Reference Standards were newly added:
(1) Anastrozole RS
(2) Budesonide RS
(3) Temozolomide RS

10. The following Reference Standards was deleted.
(1) Nartograstim RS

11. The following Reference Standards were deleted from the list of ''9.01 (2) The reference standards which are prepared by National Institute of Infectious Diseases'' and added to the list of ''9.01 (1) The reference standards which are prepared by those who have been registered to prepare them by the Minister of Health, Labour and Welfare, according to the Ministerial ordinance established by the Minister separately'':
(1) Amikacin Sulfate RS
(2) Cefaclor RS
(3) Cefalexin RS
(4) Clindamycin Phosphate RS
(5) Doxorubicin Hydrochloride RS

12. The following substances were newly added to the Official Monographs:

(1) Anastrozole
(2) Anastrozole Tablets
(3) Bicalutamide Tablets
(4) Budesonide
(5) Oxybutynin Hydrochloride
(6) Temozolomide
(7) Temozolomide Capsules
(8) Temozolomide for Injection
(9) Voglibose Orally Disintegrating Tablets
(10) Saikokeishikankyoto Extract
(11) Yokukansankachimpihange Extract

13. The following monographs were revised:

(1) Amphotericin B for Injection
(2) Amphotericin B Tablets
(3) Ampicillin Sodium and Sulbactam Sodium for Injection
(4) Benzyl Alcohol
(5) Bromhexine Hydrochloride
(6) Butropium Bromide
(7) Butyl Parahydroxybenzoate
(8) Croscarmellose Sodium
(9) Cefoperazone Sodium and Sulbactam Sodium for Injection
(10) Powdered Cellulose
(11) Enviomycin Sulfate
(12) Epoetin Beta (Genetical Recombination)
(13) Ethanol
(14) Anhydrous Ethanol
(15) Ethyl Parahydroxybenzoate
(16) Formoterol Fumarate Hydrate
(17) Glyceryl Monostearate
(18) Hypromellose Phthalate
(19) Imipenem and Cilastatin Sodium for Injection
(20) Insulin Human (Genetical Recombination)
(21) Insulin Human (Genetical Recombination) Injection
(22) Isophane Insulin Human (Genetical Recombination) Injectable Aqueous Suspension
(23) Biphasic Isophane Insulin Human (Genetical Recombination) Injectable Aqueous Suspension
(24) Magnesium Stearate
(25) D-Mannitol
(26) *dl*-Menthol
(27) *l*-Menthol
(28) Methyl Parahydroxybenzoate
(29) White Petrolatum
(30) Yellow Petrolatum
(31) Polysorbate 80
(32) Propyl Parahydroxybenzoate
(33) Sarpogrelate Hydrochloride Fine Granules
(34) Sodium Chloride
(35) Spectionomycin Hydrochloride for Injection
(36) Wheat Starch
(37) Stearic Acid
(38) Voglibose Tablets
(39) Achyranthes Root
(40) Akebia Stem
(41) Apricot Kernel
(42) Artemisia Capillaris Flower
(43) Artemisia Leaf
(44) Bearberry Leaf
(45) Bitter Cardamon
(46) Burdock Fruit
(47) Cardamon
(48) Cimicifuga Rhizome
(49) Clove
(50) Clove Oil
(51) Cnidium Monnieri Fruit
(52) Cornus Fruit
(53) Corydalis Tuber
(54) Powdered Corydalis Tuber
(55) Gardenia Fruit
(56) Ginger
(57) Powdered Ginger
(58) Glehnia Root and Rhizome
(59) Goshajinkigan Extract
(60) Goshuyuto Extract
(61) Hachimijiogan Extract
(62) Hangekobokuto Extract
(63) Keishibukuryogan Extract
(64) Leonurus Herb
(65) Magnolia Bark
(66) Maoto Extract
(67) Mukoi-Daikenchuto Extract
(68) Nutmeg
(69) Peach Kernel
(70) Powdered Peach Kernel
(71) Picrasma Wood
(72) Powdered Picrasma Wood
(73) Plantago Herb
(74) Prepared Glycyrrhiza
(75) Processed Ginger
(76) Senna Leaf
(77) Powdered Senna Leaf
(78) Shimbuto Extract
(79) Sinomenium Stem and Rhizome
(80) Tokakujokito Extract
(81) Turmeric
(82) Uncaria Hook

14. Several items of Purity were deleted from the following monographs:

(1) Acebutolol Hydrochloride

(2) Acemetacin
(3) Acetaminophen
(4) Acetazolamide
(5) Acetic Acid
(6) Glacial Acetic Acid
(7) Acetohexamide
(8) Acetylcholine Chloride for Injection
(9) Acetylcysteine
(10) Aciclovir
(11) Aclarubicin Hydrochloride
(12) Acrinol Hydrate
(13) Adrenaline
(14) Afloqualone
(15) Alacepril
(16) L-Alanine
(17) Aldioxa
(18) Alendronate Sodium Hydrate
(19) Alimemazine Tartrate
(20) Allopurinol
(21) Alprazolam
(22) Alprenolol Hydrochloride
(23) Alprostadil Injection
(24) Dried Aluminum Hydroxide Gel
(25) Aluminum Monostearate
(26) Aluminum Potassium Sulfate Hydrate
(27) Natural Aluminum Silicate
(28) Synthetic Aluminum Silicate
(29) Amantadine Hydrochloride
(30) Ambenonium Chloride
(31) Amidotrizoic Acid
(32) Amikacin Sulfate
(33) Aminophylline Hydrate
(34) Amiodarone Hydrochloride
(35) Amitriptyline Hydrochloride
(36) Amlexanox
(37) Amlodipine Besilate
(38) Ammonia Water
(39) Amobarbital
(40) Amosulalol Hydrochloride
(41) Amoxapine
(42) Amoxicillin Hydrate
(43) Anhydrous Ampicillin
(44) Ampicillin Hydrate
(45) Ampicillin Sodium
(46) Ampiroxicam
(47) Antipyrine
(48) Aprindine Hydrochloride
(49) Arbekacin Sulfate
(50) Argatroban Hydrate
(51) L-Arginine
(52) L-Arginine Hydrochloride
(53) Arotinolol Hydrochloride
(54) Ascorbic Acid
(55) L-Aspartic Acid
(56) Aspirin
(57) Aspoxicillin Hydrate
(58) Atenolol
(59) Atorvastatin Calcium Hydrate
(60) Auranofin
(61) Azathioprine
(62) Azelastine Hydrochloride
(63) Azelnidipine
(64) Azithromycin Hydrate
(65) Azosemide
(66) Aztreonam
(67) Bacampicillin Hydrochloride
(68) Bacitracin
(69) Baclofen
(70) Bamethan Sulfate
(71) Barbital
(72) Barium Sulfate
(73) Beclometasone Dipropionate
(74) Bekanamycin Sulfate
(75) Benidipine Hydrochloride
(76) Benserazide Hydrochloride
(77) Benzbromarone
(78) Benzoic Acid
(79) Benzylpenicillin Benzathine Hydrate
(80) Benzylpenicillin Potassium
(81) Bepotastine Besilate
(82) Berberine Chloride Hydrate
(83) Betahistine Mesilate
(84) Betamethasone
(85) Betamethasone Dipropionate
(86) Betamipron
(87) Betaxolol Hydrochloride
(88) Bethanechol Chloride
(89) Bezafibrate
(90) Bicalutamide
(91) Bifonazole
(92) Biotin
(93) Biperiden Hydrochloride
(94) Bisacodyl
(95) Bismuth Subgallate
(96) Bisoprolol Fumarate
(97) Bleomycin Hydrochloride
(98) Bleomycin Sulfate
(99) Boric Acid
(100) Bromazepam
(101) Bromfenac Sodium Hydrate
(102) Bromhexine Hydrochloride
(103) Bromocriptine Mesilate
(104) Bromovalerylurea
(105) Brotizolam
(106) Bucillamine
(107) Bucumolol Hydrochloride

(108) Bufetolol Hydrochloride
(109) Buformin Hydrochloride
(110) Bumetanide
(111) Bunazosin Hydrochloride
(112) Bupivacaine Hydrochloride Hydrate
(113) Bupranolol Hydrochloride
(114) Buprenorphine Hydrochloride
(115) Busulfan
(116) Butenafine Hydrochloride
(117) Butropium Bromide
(118) Butyl Parahydroxybenzoate
(119) Cabergoline
(120) Cadralazine
(121) Anhydrous Caffeine
(122) Caffeine Hydrate
(123) Caffeine and Sodium Benzoate
(124) Precipitated Calcium Carbonate
(125) Calcium Chloride Hydrate
(126) Calcium Folinate Hydrate
(127) Calcium Gluconate Hydrate
(128) Calcium Hydroxide
(129) Calcium Lactate Hydrate
(130) Calcium Levofolinate Hydrate
(131) Calcium Pantothenate
(132) Calcium Paraaminosalicylate Hydrate
(133) Anhydrous Dibasic Calcium Phosphate
(134) Dibasic Calcium Phosphate Hydrate
(135) Monobasic Calcium Phosphate Hydrate
(136) Calcium Polystyrene Sulfonate
(137) Calcium Sodium Edetate Hydrate
(138) Calcium Stearate
(139) Camostat Mesilate
(140) Candesartan Cilexetil
(141) Captopril
(142) Carbamazepine
(143) Carbazochrome Sodium Sulfonate Hydrate
(144) Carbidopa Hydrate
(145) L-Carbocisteine
(146) Carmellose
(147) Carmellose Calcium
(148) Carmellose Sodium
(149) Croscarmellose Sodium
(150) Carmofur
(151) Carteolol Hydrochloride
(152) Carumonam Sodium
(153) Carvedilol
(154) Cefaclor
(155) Cefadroxil
(156) Cefalexin
(157) Cefalotin Sodium
(158) Cefatrizine Propylene Glycolate
(159) Cefazolin Sodium
(160) Cefazolin Sodium Hydrate
(161) Cefbuperazone Sodium
(162) Cefcapene Pivoxil Hydrochloride Hydrate
(163) Cefdinir
(164) Cefditoren Pivoxil
(165) Cefepime Dihydrochloride Hydrate
(166) Cefmenoxime Hydrochloride
(167) Cefmetazole Sodium
(168) Cefminox Sodium Hydrate
(169) Cefodizime Sodium
(170) Cefoperazone Sodium
(171) Cefotaxime Sodium
(172) Cefotetan
(173) Cefotiam Hexetil Hydrochloride
(174) Cefotiam Hydrochloride
(175) Cefozopran Hydrochloride
(176) Cefpiramide Sodium
(177) Cefpirome Sulfate
(178) Cefpodoxime Proxetil
(179) Cefroxadine Hydrate
(180) Cefsulodin Sodium
(181) Ceftazidime Hydrate
(182) Cefteram Pivoxil
(183) Ceftibuten Hydrate
(184) Ceftizoxime Sodium
(185) Ceftriaxone Sodium Hydrate
(186) Cefuroxime Axetil
(187) Celecoxib
(188) Cellacefate
(189) Microcrystalline Cellulose
(190) Powdered Cellulose
(191) Cetirizine Hydrochloride
(192) Cetotiamine Hydrochloride Hydrate
(193) Cetraxate Hydrochloride
(194) Chenodeoxycholic Acid
(195) Chloramphenicol
(196) Chloramphenicol Palmitate
(197) Chloramphenicol Sodium Succinate
(198) Chlordiazepoxide
(199) Chlorhexidine Hydrochloride
(200) Chlormadinone Acetate
(201) Chlorphenesin Carbamate
(202) Chlorpheniramine Maleate
(203) *d*-Chlorpheniramine Maleate
(204) Chlorpromazine Hydrochloride
(205) Chlorpropamide
(206) Cibenzoline Succinate
(207) Ciclacillin
(208) Ciclosporin
(209) Cilastatin Sodium
(210) Cilazapril Hydrate
(211) Cilnidipine
(212) Cilostazol
(213) Cimetidine

(214) Cinoxacin
(215) Ciprofloxacin
(216) Ciprofloxacin Hydrochloride Hydrate
(217) Citicoline
(218) Anhydrous Citric Acid
(219) Citric Acid Hydrate
(220) Clarithromycin
(221) Clebopride Malate
(222) Clemastine Fumarate
(223) Clindamycin Hydrochloride
(224) Clindamycin Phosphate
(225) Clinofibrate
(226) Clobetasol Propionate
(227) Clocapramine Hydrochloride Hydrate
(228) Clofedanol Hydrochloride
(229) Clofibrate
(230) Clomifene Citrate
(231) Clomipramine Hydrochloride
(232) Clonazepam
(233) Clonidine Hydrochloride
(234) Cloperastine Fendizoate
(235) Cloperastine Hydrochloride
(236) Clopidogrel Sulfate
(237) Clorazepate Dipotassium
(238) Clotiazepam
(239) Clotrimazole
(240) Cloxacillin Sodium Hydrate
(241) Cloxazolam
(242) Colestimide
(243) Colistin Sodium Methanesulfonate
(244) Copovidone
(245) Croconazole Hydrochloride
(246) Crospovidone
(247) Cyanamide
(248) Cyclopentolate Hydrochloride
(249) Cyclophosphamide Hydrate
(250) Cycloserine
(251) Cyproheptadine Hydrochloride Hydrate
(252) L-Cysteine
(253) L-Cysteine Hydrochloride Hydrate
(254) L-Cystine
(255) Cytarabine
(256) Danazol
(257) Dantrolene Sodium Hydrate
(258) Daunorubicin Hydrochloride
(259) Deferoxamine Mesilate
(260) Dehydrocholic Acid
(261) Purified Dehydrocholic Acid
(262) Dehydrocholic Acid Injection
(263) Demethylchlortetracycline Hydrochloride
(264) Dexamethasone
(265) Dextran 40
(266) Dextran 70
(267) Dextran Sulfate Sodium Sulfur 5
(268) Dextran Sulfate Sodium Sulfur 18
(269) Dextrin
(270) Dextromethorphan Hydrobromide Hydrate
(271) Diazepam
(272) Dibekacin Sulfate
(273) Dibucaine Hydrochloride
(274) Diclofenac Sodium
(275) Diethylcarbamazine Citrate
(276) Difenidol Hydrochloride
(277) Diflorasone Diacetate
(278) Diflucortolone Valerate
(279) Dihydroergotoxine Mesilate
(280) Dilazep Hydrochloride Hydrate
(281) Diltiazem Hydrochloride
(282) Dimemorfan Phosphate
(283) Dimercaprol
(284) Dimorpholamine
(285) Diphenhydramine
(286) Diphenhydramine Hydrochloride
(287) Diphenhydramine Tannate
(288) Dipyridamole
(289) Disopyramide
(290) Distigmine Bromide
(291) Disulfiram
(292) Dobutamine Hydrochloride
(293) Docetaxel Hydrate
(294) Domperidone
(295) Donepezil Hydrochloride
(296) Dopamine Hydrochloride
(297) Doripenem Hydrate
(298) Dorzolamide Hydrochloride
(299) Doxapram Hydrochloride Hydrate
(300) Doxazosin Mesilate
(301) Doxifluridine
(302) Doxycycline Hydrochloride Hydrate
(303) Droperidol
(304) Droxidopa
(305) Dydrogesterone
(306) Ebastine
(307) Ecabet Sodium Hydrate
(308) Ecothiopate Iodide
(309) Edaravone
(310) Edrophonium Chloride
(311) Emedastine Fumarate
(312) Emorfazone
(313) Enalapril Maleate
(314) Enoxacin Hydrate
(315) Entacapone
(316) Enviomycin Sulfate
(317) Epalrestat
(318) Eperisone Hydrochloride
(319) Ephedrine Hydrochloride

(320) Epirizole
(321) Epirubicin Hydrochloride
(322) Eplerenone
(323) Eribulin Mesilate
(324) Erythromycin
(325) Estazolam
(326) Estriol
(327) Etacrynic Acid
(328) Ethambutol Hydrochloride
(329) Ethenzamide
(330) Ethionamide
(331) Ethosuximide
(332) Ethyl Aminobenzoate
(333) Ethylcellulose
(334) Ethyl L-Cysteine Hydrochloride
(335) Ethylenediamine
(336) Ethyl Icosapentate
(337) Ethyl Loflazepate
(338) Ethyl Parahydroxybenzoate
(339) Etidronate Disodium
(340) Etilefrine Hydrochloride
(341) Etizolam
(342) Etodolac
(343) Etoposide
(344) Famotidine
(345) Faropenem Sodium Hydrate
(346) Felbinac
(347) Felodipine
(348) Fenbufen
(349) Fenofibrate
(350) Fentanyl Citrate
(351) Ferrous Sulfate Hydrate
(352) Fexofenadine Hydrochloride
(353) Flavin Adenine Dinucleotide Sodium
(354) Flavoxate Hydrochloride
(355) Flecainide Acetate
(356) Flomoxef Sodium
(357) Flopropione
(358) Fluconazole
(359) Flucytosine
(360) Fludiazepam
(361) Fludrocortisone Acetate
(362) Flunitrazepam
(363) Fluorometholone
(364) Fluorouracil
(365) Fluphenazine Enanthate
(366) Flurazepam Hydrochloride
(367) Flurbiprofen
(368) Flutamide
(369) Flutoprazepam
(370) Fluvoxamine Maleate
(371) Formoterol Fumarate Hydrate
(372) Fosfomycin Calcium Hydrate
(373) Fosfomycin Sodium
(374) Fradiomycin Sulfate
(375) Fructose
(376) Fructose Injection
(377) Fudosteine
(378) Furosemide
(379) Fursultiamine Hydrochloride
(380) Gabexate Mesilate
(381) β-Galactosidase (Aspergillus)
(382) β-Galactosidase (Penicillium)
(383) Gatifloxacin Hydrate
(384) Gefarnate
(385) Gefitinib
(386) Gelatin
(387) Purified Gelatin
(388) Gentamicin Sulfate
(389) Glibenclamide
(390) Gliclazide
(391) Glimepiride
(392) Glucose
(393) Glucose Hydrate
(394) Purified Glucose
(395) L-Glutamic Acid
(396) L-Glutamine
(397) Glutathione
(398) Glycerin
(399) Concentrated Glycerin
(400) Glycine
(401) Guaifenesin
(402) Guanabenz Acetate
(403) Guanethidine Sulfate
(404) Haloperidol
(405) Haloxazolam
(406) Heparin Calcium
(407) Heparin Sodium
(408) Heparin Sodium Injection
(409) L-Histidine
(410) L-Histidine Hydrochloride Hydrate
(411) Homochlorcyclizine Hydrochloride
(412) Hydralazine Hydrochloride
(413) Hydrochloric Acid
(414) Dilute Hydrochloric Acid
(415) Hydrochlorothiazide
(416) Hydrocortisone Butyrate
(417) Hydrocortisone Sodium Phosphate
(418) Hydrocotarnine Hydrochloride Hydrate
(419) Hydrogenated Oil
(420) Hydroxyethylcellulose
(421) Hydroxypropylcellulose
(422) Low Substituted Hydroxypropylcellulose
(423) Hydroxyzine Hydrochloride
(424) Hydroxyzine Pamoate
(425) Hymecromone

(426) Hypromellose
(427) Hypromellose Acetate Succinate
(428) Hypromellose Phthalate
(429) Ibudilast
(430) Ibuprofen
(431) Ibuprofen Piconol
(432) Idarubicin Hydrochloride
(433) Idoxuridine
(434) Ifenprodil Tartrate
(435) Imidapril Hydrochloride
(436) Imipenem Hydrate
(437) Indapamide
(438) Indenolol Hydrochloride
(439) Indigocarmine
(440) Indometacin
(441) Iohexol
(442) Iopamidol
(443) Iotalamic Acid
(444) Iotroxic Acid
(445) Ipratropium Bromide Hydrate
(446) Ipriflavone
(447) Irbesartan
(448) Irinotecan Hydrochloride Hydrate
(449) Irsogladine Maleate
(450) Isepamicin Sulfate
(451) L-Isoleucine
(452) Isomalt Hydrate
(453) Isoniazid
(454) *l*-Isoprenaline Hydrochloride
(455) Isopropylantipyrine
(456) Isosorbide
(457) Isosorbide Dinitrate
(458) Isosorbide Mononitrate 70%/Lactose 30%
(459) Isoxsuprine Hydrochloride
(460) Itraconazole
(461) Josamycin
(462) Josamycin Propionate
(463) Kainic Acid Hydrate
(464) Kanamycin Monosulfate
(465) Kanamycin Sulfate
(466) Ketamine Hydrochloride
(467) Ketoconazole
(468) Ketoprofen
(469) Ketotifen Fumarate
(470) Kitasamycin Tartrate
(471) Labetalol Hydrochloride
(472) Lactic Acid
(473) L-Lactic Acid
(474) Anhydrous Lactose
(475) Lactose Hydrate
(476) Lactulose
(477) Lafutidine
(478) Lanoconazole
(479) Lansoprazole
(480) Latamoxef Sodium
(481) Lenampicillin Hydrochloride
(482) L-Leucine
(483) Levallorphan Tartrate
(484) Levodopa
(485) Levofloxacin Hydrate
(486) Levomepromazine Maleate
(487) Lidocaine
(488) Lincomycin Hydrochloride Hydrate
(489) Lisinopril Hydrate
(490) Lithium Carbonate
(491) Lobenzarit Sodium
(492) Lorazepam
(493) Losartan Potassium
(494) Loxoprofen Sodium Hydrate
(495) L-Lysine Acetate
(496) L-Lysine Hydrochloride
(497) Lysozyme Hydrochloride
(498) Magnesium Aluminosilicate
(499) Magnesium Aluminometasilicate
(500) Magnesium Carbonate
(501) Magnesium Oxide
(502) Magnesium Stearate
(503) Magnesium Sulfate Hydrate
(504) Maltose Hydrate
(505) Manidipine Hydrochloride
(506) D-Mannitol
(507) Maprotiline Hydrochloride
(508) Meclofenoxate Hydrochloride
(509) Medazepam
(510) Medicinal Carbon
(511) Medicinal Soap
(512) Medroxyprogesterone Acetate
(513) Mefenamic Acid
(514) Mefloquine Hydrochloride
(515) Mefruside
(516) Meglumine
(517) Melphalan
(518) Menatetrenone
(519) Mepenzolate Bromide
(520) Mepitiostane
(521) Mepivacaine Hydrochloride
(522) Mequitazine
(523) Mercaptopurine Hydrate
(524) Meropenem Hydrate
(525) Mesalazine
(526) Mestranol
(527) Metenolone Acetate
(528) Metenolone Enanthate
(529) Metformin Hydrochloride
(530) L-Methionine
(531) Methoxsalen

(532) Methylcellulose
(533) Methyldopa Hydrate
(534) *dl*-Methylephedrine Hydrochloride
(535) Methyl Parahydroxybenzoate
(536) Methylprednisolone Succinate
(537) Methyl Salicylate
(538) Meticrane
(539) Metildigoxin
(540) Metoclopramide
(541) Metoprolol Tartrate
(542) Metronidazole
(543) Metyrapone
(544) Mexiletine Hydrochloride
(545) Miconazole
(546) Miconazole Nitrate
(547) Micronomicin Sulfate
(548) Midecamycin
(549) Midecamycin Acetate
(550) Miglitol
(551) Migrenin
(552) Minocycline Hydrochloride
(553) Mitiglinide Calcium Hydrate
(554) Mizoribine
(555) Montelukast Sodium
(556) Mosapride Citrate Hydrate
(557) Mupirocin Calcium Hydrate
(558) Nabumetone
(559) Nadolol
(560) Nafamostat Mesilate
(561) Naftopidil
(562) Nalidixic Acid
(563) Naphazoline Nitrate
(564) Naproxen
(565) Nateglinide
(566) Nicardipine Hydrochloride
(567) Nicergoline
(568) Niceritrol
(569) Nicomol
(570) Nicorandil
(571) Nicotinamide
(572) Nicotinic Acid
(573) Nifedipine
(574) Nilvadipine
(575) Nitrazepam
(576) Nitrendipine
(577) Nizatidine
(578) Norfloxacin
(579) Norgestrel
(580) Nortriptyline Hydrochloride
(581) Noscapine
(582) Nystatin
(583) Ofloxacin
(584) Olmesartan Medoxomil
(585) Olopatadine Hydrochloride
(586) Omeprazole
(587) Orciprenaline Sulfate
(588) Oxapium Iodide
(589) Oxaprozin
(590) Oxazolam
(591) Oxethazaine
(592) Oxprenolol Hydrochloride
(593) Oxybuprocaine Hydrochloride
(594) Oxydol
(595) Oxytetracycline Hydrochloride
(596) Ozagrel Sodium
(597) Panipenem
(598) Pantethine
(599) Paraffin
(600) Liquid Paraffin
(601) Light Liquid Paraffin
(602) Parnaparin Sodium
(603) Paroxetine Hydrochloride Hydrate
(604) Pazufloxacin Mesilate
(605) Pemirolast Potassium
(606) Penbutolol Sulfate
(607) Pentazocine
(608) Pentobarbital Calcium
(609) Pentoxyverine Citrate
(610) Peplomycin Sulfate
(611) Perphenazine
(612) Perphenazine Maleate
(613) White Petrolatum
(614) Yellow Petrolatum
(615) Phenethicillin Potassium
(616) Phenobarbital
(617) L-Phenylalanine
(618) Phenylbutazone
(619) Phenytoin
(620) Phenytoin Sodium for Injection
(621) Phytonadione
(622) Pilsicainide Hydrochloride Hydrate
(623) Pimaricin
(624) Pimozide
(625) Pindolol
(626) Pioglitazone Hydrochloride
(627) Pipemidic Acid Hydrate
(628) Piperacillin Hydrate
(629) Piperacillin Sodium
(630) Piperazine Adipate
(631) Piperazine Phosphate Hydrate
(632) Pirarubicin
(633) Pirenoxine
(634) Pirenzepine Hydrochloride Hydrate
(635) Piroxicam
(636) Pitavastatin Calcium Hydrate
(637) Pivmecillinam Hydrochloride

(638) Polaprezinc
(639) Polymixin B Sulfate
(640) Polyoxyl 40 Stearate
(641) Polysorbate 80
(642) Potassium Bromide
(643) Potassium Canrenoate
(644) Potassium Carbonate
(645) Potassium Chloride
(646) Potassium Clavulanate
(647) Potassium Hydroxide
(648) Potassium Iodide
(649) Potassium Permanganate
(650) Potassium Sulfate
(651) Povidone
(652) Povidone-Iodine
(653) Pranlukast Hydrate
(654) Pranoprofen
(655) Prasterone Sodium Sulfate Hydrate
(656) Pravastatin Sodium
(657) Prazepam
(658) Prazosin Hydrochloride
(659) Prednisolone
(660) Prednisolone Sodium Phosphate
(661) Primidone
(662) Probenecid
(663) Probucol
(664) Procainamide Hydrochloride
(665) Procaine Hydrochloride
(666) Procarbazine Hydrochloride
(667) Procaterol Hydrochloride Hydrate
(668) Prochlorperazine Maleate
(669) Proglumide
(670) L-Proline
(671) Promethazine Hydrochloride
(672) Propafenone Hydrochloride
(673) Propiverine Hydrochloride
(674) Propranolol Hydrochloride
(675) Propylene Glycol
(676) Propyl Parahydroxybenzoate
(677) Prothionamide
(678) Protirelin
(679) Protirelin Tartrate Hydrate
(680) Pullulan
(681) Pyrantel Pamoate
(682) Pyrazinamide
(683) Pyridostigmine Bromide
(684) Pyridoxal Phosphate Hydrate
(685) Pyridoxine Hydrochloride
(686) Quetiapine Fumarate
(687) Quinapril Hydrochloride
(688) Quinine Ethyl Carbonate
(689) Quinine Sulfate Hydrate
(690) Rabeprazole Sodium
(691) Ranitidine Hydrochloride
(692) Rebamipide
(693) Ribavirin
(694) Riboflavin Butyrate
(695) Ribostamycin Sulfate
(696) Rifampicin
(697) Rilmazafone Hydrochloride Hydrate
(698) Ringer's Solution
(699) Risperidone
(700) Ritodrine Hydrochloride
(701) Rosuvastatin Calcium
(702) Roxatidine Acetate Hydrochloride
(703) Roxithromycin
(704) Saccharin
(705) Saccharin Sodium Hydrate
(706) Salazosulfapyridine
(707) Salbutamol Sulfate
(708) Salicylic Acid
(709) Sarpogrelate Hydrochloride
(710) Scopolamine Butylbromide
(711) L-Serine
(712) Purified Shellac
(713) White Shellac
(714) Light Anhydrous Silicic Acid
(715) Silodosin
(716) Silver Nitrate
(717) Simvastatin
(718) Sitagliptin Phosphate Hydrate
(719) Sivelestat Sodium Hydrate
(720) Sodium Acetate Hydrate
(721) Sodium Aurothiomalate
(722) Sodium Benzoate
(723) Sodium Bicarbonate
(724) Sodium Bisulfite
(725) Sodium Borate
(726) Sodium Bromide
(727) Dried Sodium Carbonate
(728) Sodium Carbonate Hydrate
(729) Sodium Chloride
(730) Isotonic Sodium Chloride Solution
(731) Sodium Citrate Hydrate
(732) Sodium Cromoglicate
(733) Disodium Edetate Hydrate
(734) Sodium Fusidate
(735) Purified Sodium Hyaluronate
(736) Sodium Hydroxide
(737) Sodium Iodide
(738) Sodium L-Lactate Solution
(739) Sodium L-Lactate Ringer's Solution
(740) Dibasic Sodium Phosphate Hydrate
(741) Sodium Picosulfate Hydrate
(742) Sodium Polystyrene Sulfonate
(743) Sodium Pyrosulfite

(744) Sodium Risedronate Hydrate
(745) Sodium Salicylate
(746) Sodium Starch Glycolate
(747) Dried Sodium Sulfite
(748) Sodium Thiosulfate Hydrate
(749) Sodium Valproate
(750) Sorbitan Sesquioleate
(751) D-Sorbitol
(752) D-Sorbitol Solution
(753) Spiramycin Acetate
(754) Stearic Acid
(755) Streptomycin Sulfate
(756) Sucralfate Hydrate
(757) White Soft Sugar
(758) Sulbactam Sodium
(759) Sulbenicillin Sodium
(760) Sulfamethizole
(761) Sulfamethoxazole
(762) Sulfamonomethoxine Hydrate
(763) Sulfisoxazole
(764) Sulfobromophthalein Sodium
(765) Sulfur
(766) Sulindac
(767) Sulpiride
(768) Sulpyrine Hydrate
(769) Sultamicillin Tosilate Hydrate
(770) Sultiame
(771) Tacrolimus Hydrate
(772) Talampicillin Hydrochloride
(773) Taltirelin Hydrate
(774) Tamoxifen Citrate
(775) Tamsulosin Hydrochloride
(776) Tartaric Acid
(777) Taurine
(778) Tazobactam
(779) Tegafur
(780) Teicoplanin
(781) Telmisartan
(782) Temocapril Hydrochloride
(783) Teprenone
(784) Terbinafine Hydrochloride
(785) Terbutaline Sulfate
(786) Tetracaine Hydrochloride
(787) Tetracycline Hydrochloride
(788) Theophylline
(789) Thiamazole
(790) Thiamine Chloride Hydrochloride
(791) Thiamine Nitrate
(792) Thiamylal Sodium
(793) Thiopental Sodium
(794) Thiopental Sodium for Injection
(795) Thioridazine Hydrochloride
(796) L-Threonine
(797) Tiapride Hydrochloride
(798) Tiaramide Hydrochloride
(799) Ticlopidine Hydrochloride
(800) Timepidium Bromide Hydrate
(801) Timolol Maleate
(802) Tinidazole
(803) Tipepidine Hibenzate
(804) Tizanidine Hydrochloride
(805) Tobramycin
(806) Tocopherol
(807) Tocopherol Acetate
(808) Tocopherol Nicotinate
(809) Todralazine Hydrochloride Hydrate
(810) Tofisopam
(811) Tolbutamide
(812) Tolnaftate
(813) Tolperisone Hydrochloride
(814) Tosufloxacin Tosilate Hydrate
(815) Tramadol Hydrochloride
(816) Tranexamic Acid
(817) Tranilast
(818) Trapidil
(819) Trehalose Hydrate
(820) Trepibutone
(821) Triamcinolone
(822) Triamcinolone Acetonide
(823) Triamterene
(824) Triazolam
(825) Trichlormethiazide
(826) Triclofos Sodium
(827) Trientine Hydrochloride
(828) Trihexyphenidyl Hydrochloride
(829) Trimebutine Maleate
(830) Trimetazidine Hydrochloride
(831) Trimethadione
(832) Trimetoquinol Hydrochloride Hydrate
(833) Tropicamide
(834) Troxipide
(835) L-Tryptophan
(836) Tulobuterol
(837) Tulobuterol Hydrochloride
(838) L-Tyrosine
(839) Ubenimex
(840) Ubidecarenone
(841) Ulinastatin
(842) Urapidil
(843) Urea
(844) Urokinase
(845) Ursodeoxycholic Acid
(846) Valaciclovir Hydrochloride
(847) L-Valine
(848) Valsartan
(849) Vancomycin Hydrochloride

(850) Verapamil Hydrochloride
(851) Voglibose
(852) Voriconazole
(853) Warfarin Potassium
(854) Wine
(855) Xylitol
(856) Zaltoprofen
(857) Zidovudine
(858) Zinc Chloride
(859) Zinc Oxide
(860) Zinc Sulfate Hydrate
(861) Zolpidem Tartrate
(862) Zonisamide
(863) Zopiclone

15. The following monographs were deleted:
(1) Nartograstim (Genetical Recombination)
(2) Nartograstim for Injection (Genetical Recombination)

16. The following articles were newly added to Ultraviolet-visible Reference Spectra:
(1) Anastrozole
(2) Budesonide
(3) Oxybutynine Hydrochloride
(4) Temozolomide

17. The following articles were newly added to Infrared Reference Spectra:
(1) Anastrozole
(2) Budesonide
(3) Croscarmellose Sodium
(4) Oxybutynine Hydrochloride
(5) White Petrolatum
(6) Yellow Petrolatum
(7) Temozolomide

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Supplement I to The Japanese Pharmacopoeia Eighteenth Edition

GENERAL TESTS, PROCESSES AND APPARATUS

Add the following:

2.00 Chromatography

This test is harmonized with the European Pharmacopoeia and the U.S. Pharmacopeia.

The corresponding part of the attributes/provisions which are agreed as non-harmonized within the scope of the harmonization is marked with symbols (◆ ◆) and the corresponding parts which are agreed as the JP local requirement other than the scope of the harmonization are marked with symbols (◇ ◇).

Information on the harmonization with the European Pharmacopoeia and the U.S. Pharmacopeia is available on the website of the Pharmaceuticals and Medical Devices Agency.

1. Introduction

Chromatographic separation techniques are multi-stage separation procedures in which the components of a sample are distributed between 2 phases, one of which is stationary, while the other is mobile. The stationary phase may be a solid or a liquid supported on a solid or a gel. The stationary phase may be packed in a column, spread as a layer, or distributed as a film, etc. The mobile phase may be gaseous or liquid or supercritical fluid. The separation may be based on adsorption, mass distribution (partition), ion exchange, etc., or may be based on differences in the physico-chemical properties of the molecules such as size, mass, volume, etc. This chapter contains definitions and calculations of common parameters and generally applicable requirements for system suitability. ◇The prescription described in Liquid Chromatography <2.01> other than the prescription of this test can be applied to the system suitability of liquid chromatography.◇ Principles of separation, apparatus and methods are given in the corresponding general tests.

2. Definitions

The system suitability and acceptance criteria in monographs have been set using parameters as defined below. With some equipment, certain parameters, such as the signal-to-noise ratio and resolution, can be calculated using software provided by the manufacturer. It is the responsibility of the user to ensure that the calculation methods used in the software are equivalent to the requirements of the Japanese Pharmacopoeia and to make any necessary corrections if this is not the case.

Chromatogram

A graphical or other representation of detector response, effluent concentration or other quantity used as a measure of effluent concentration, versus time or volume. Idealized chromatograms are represented as a sequence of Gaussian peaks on a baseline (Fig. 2.00-1).

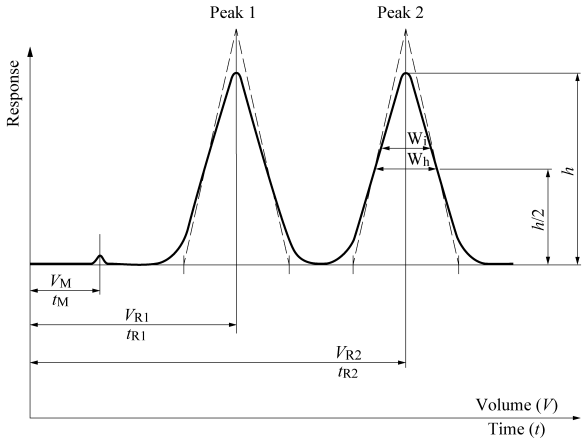


Fig. 2.00-1

V_M: Hold-up volume
t_M: Hold-up time
V_{R1}: Retention volume of peak 1
t_{R1}: Retention time of peak 1
V_{R2}: Retention volume of peak 2
t_{R2}: Retention time of peak 2
W_h: Peak width at half-height
W_i: Peak width at the inflexion point
h: Height of the peak
$h/2$: Half-height of peak

Distribution constant (K_0)

In size-exclusion chromatography, the elution characteristics of a component in a particular column may be given by the distribution constant (also referred to as distribution coefficient), which is calculated using the following equation:

$$K_0 = \frac{t_R - t_0}{t_t - t_0}$$

t_R: Retention time
t_0: Retention time of an unretained compound
t_t: Total mobile phase time

Dwell volume (D) (also referred to as V_D):

The dwell volume (also known as gradient delay volume) is the volume between the point at which the eluents meet and the inlet of the column. It can be determined using the following procedure.

Column: replace the chromatographic column by an appropriate capillary tubing (e.g. 1 m × 0.12 mm).

Mobile phase.

Mobile phase A: water.
Mobile phase B: 0.1 vol% solution of acetone in water.

Time (min)	Mobile phase A (vol%)	Mobile phase B (vol%)
0 – 20	100 → 0	0 → 100
20 – 30	0	100

Flow rate: Set to obtain sufficient back-pressure (e.g. 2 mL/min).

Detection: Spectrophotometer at 265 nm.

Determine the time ($t_{0.5}$) (minutes) when the absorbance has increased by 50% (Fig. 2.00-2).

$$D = t_D \times F$$

t_D: $t_{0.5} - 0.5\ t_G$ (min)
t_G: Pre-defined gradient time (= 20 min)
F: Flow rate (mL/min)

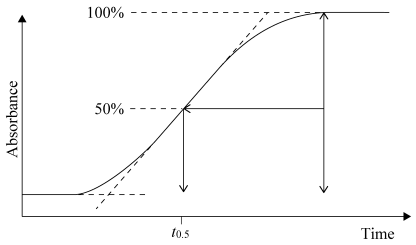


Fig. 2.00-2

Note: Where applicable, this measurement is performed with the autosampler in the inject position so as to include the injection loop volume in the dwell volume.

Hold-up time (t_M)

Time required for elution of an unretained component (Fig. 2.00-1, baseline scale being in minutes or seconds).

In size-exclusion chromatography, the term retention time of an unretained compound (t_0) is used.

Hold-up volume(V_M)

Volume of the mobile phase required for elution of an unretained component. It may be calculated from the hold-up time and the flow rate (F) in mL/minute using the following equation:

$$V_M = t_M \times F$$

In size-exclusion chromatography, the term retention volume of an unretained compound (V_0) is used.

Peak

Portion of a chromatogram recording the detector response when a single component (or 2 or more unresolved components) is eluted from the column.

The peak response may be represented by the peak area or the peak height (h).

Peak-to-valley ratio (p/v)

The peak-to-valley ratio may be employed as a system suitability criterion when baseline separation between two peaks is not achieved (Fig. 2.00-3).

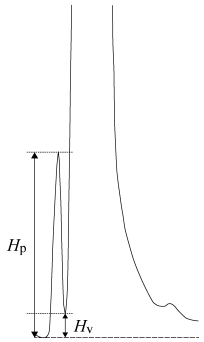


Fig. 2.00-3

$$p/v = \frac{H_p}{H_v}$$

H_p: Height above the extrapolated baseline of the minor peak
H_v: Height above the extrapolated baseline at the lowest point of the curve separating the minor and major peaks

Plate height (H) (synonym: Height equivalent to one theoretical plate (HETP))

Ratio of the column length (L) (μm) to the plate number (N):

$$H = \frac{L}{N}$$

Plate number (N)

A number indicative of column performance (column efficiency). It can only be calculated from data obtained under either isothermal, isocratic or isodense conditions, depending on the technique, as the plate number, using the following equation, the values of t_R and w_h being expressed in the same units.

$$N = 5.54\left(\frac{t_R}{w_h}\right)^2$$

t_R: Retention time of the peak corresponding to the component;
w_h: Peak width at half-height ($h/2$).

The plate number varies with the component as well as with the column, the column temperature, the mobile phase and the retention time.

Reduced plate height (h)

Ratio of the plate height (H) (μm) to the particle diameter (d_p) (μm):

$$h = \frac{H}{d_p}$$

Relative retardation (R_{rel})

The relative retardation, used in thin-layer chromatography, is calculated as the ratio of the distances travelled by the spot of the compound of interest and a reference com-

pound (Fig. 2.00-4).

$$R_{rel} = b/c$$

a: Migration distance of the mobile phase
b: Migration distance of the compound of interest
c: Migration distance of the reference compound

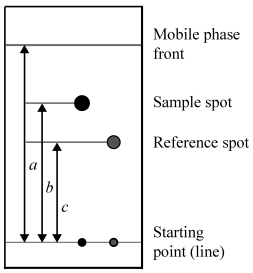


Fig. 2.00-4

Relative retention (r)

Relative retention is calculated as an estimate using the following equation:

$$r = \frac{t_{Ri} - t_M}{t_{Rst} - t_M}$$

t_{Ri}: Retention time of the peak of the component of interest
t_{Rst}: Retention time of the reference peak (usually the peak corresponding to the substance to be examined)
t_M: Hold-up time

Relative retention, unadjusted (r_G) or (RRT)

Unadjusted relative retention is calculated using the following equation:

$$r_G = \frac{t_{Ri}}{t_{Rst}}$$

Unless otherwise indicated, values for relative retention stated in monographs correspond to unadjusted relative retention.

Relative retention time (RRT):

see Relative retention, unadjusted.

Resolution (R_S)

The resolution between peaks of 2 components (Fig. 2.00-1) may be calculated using the following equation:

$$R_S = \frac{1.18\ (t_{R2} - t_{R1})}{w_{h1} + w_{h2}}$$

t_{R1}, t_{R2}: Retention times of the peaks, $t_{R2} > t_{R1}$
w_{h1}, w_{h2}: Peak widths at half-height

◇Complete separation means the resolution of not less than 1.5, and is also referred to as baseline separation.◇

In quantitative thin-layer chromatography, using densitometry, the migration distances are used instead of retention times and the resolution between peaks of 2 components may be calculated using the following equation:

$$R_S = \frac{1.18a\ (R_{F2} - R_{F1})}{w_{h1} + w_{h2}}$$

$R_{F2} > R_{F1}$
R_{F1}, R_{F2}: Retardation factors of the peaks
w_{h1}, w_{h2}: Peak widths at half-height
a: Migration distance of the solvent front

Retardation factor (R_F)

The retardation factor, used in thin-layer chromatography, is the ratio of the distance from the point of application to the center of the spot and the distance simultaneously travelled by the solvent front from the point of application (Fig. 2.00-4).

$$R_F = \frac{b}{a}$$

b: Migration distance of the compound of interest
a: Migration distance of the solvent front

Retention factor (k)

The retention factor (also known as mass distribution ratio (D_m) or capacity factor (k')) is defined as:

$$k = \frac{\text{amount of component in stationary phase}}{\text{amount of component in mobile phase}} = K_C \frac{V_S}{V_M}$$

K_C: Distribution constant (also known as equilibrium distribution coefficient);
V_S: Volume of the stationary phase
V_M: Volume of the mobile phase

The retention factor of a component may be determined from the chromatogram using the following equation:

$$k = \frac{t_R - t_M}{t_M}$$

t_R: Retention time
t_M: Hold-up time

Retention time (t_R)

Time elapsed between the injection of the sample and the appearance of the maximum peak response of the eluted sample zone (Fig. 2.00-1, baseline scale being in minutes or seconds).

Retention volume (V_R)

Volume of the mobile phase required for elution of a component. It may be calculated from the retention time and the flow rate (F: mL/minute) using the following equation:

$$V_R = t_R \times F$$

Retention time of an unretained compound (t_0)

In size-exclusion chromatography, retention time of a component whose molecules are larger than the largest gel pores (Fig. 2.00-5).

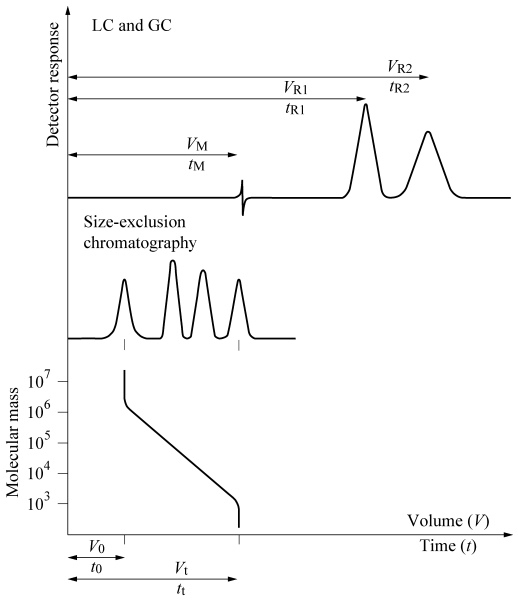


Fig. 2.00-5

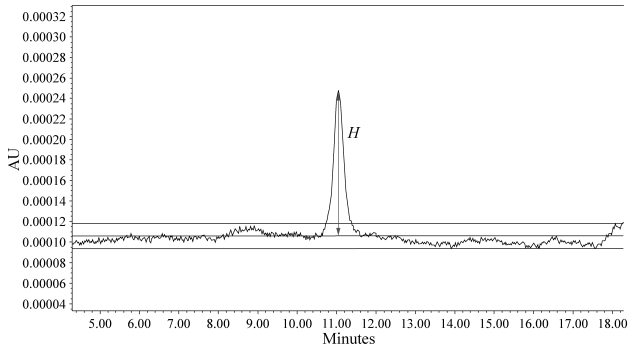


Fig. 2.00-6. Chromatogram of the reference solution

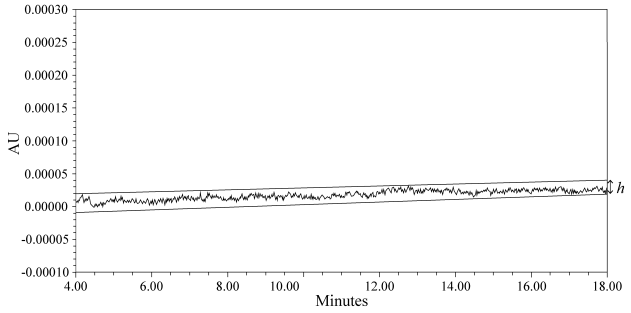


Fig. 2.00-7. Chromatogram of a blank

Retention volume of an unretained compound (V_0)

In size-exclusion chromatography, retention volume of a component whose molecules are larger than the largest gel pores. It may be calculated from the retention time of an unretained compound and the flow rate (F: mL/minute) using the following equation:

$$V_0 = t_0 \times F$$

Separation factor (α)

Relative retention calculated for two adjacent peaks (by convention, the value of the separation factor is always > 1):

$$\alpha = k_2/k_1$$

k_1: Retention factor of the first peak

k_2: Retention factor of the second peak

Signal-to-noise ratio (S/N)

The short-term noise influences the precision and accuracy of quantitation. The signal-to-noise ratio is calculated using the following equation:

$$S/N = \frac{2H}{h}$$

H: Height of the peak (Fig. 2.00-6) corresponding to the component concerned, in the chromatogram obtained with the prescribed reference solution, measured from the maximum of the peak to the extrapolated baseline of the signal observed over a distance equal to 20 times the width at half-height

h: Range of the noise in a chromatogram obtained after injection of a blank (Fig. 2.00-7), observed over a distance equal to 20 times the width at half-height of the peak in the chromatogram obtained with the prescribed reference solution and, if possible, situated equally around the place where this peak would be found.

If a baseline of 20 times the width at half-height is not obtainable because of peaks due to the solvents or reagents, or arising from the mobile phase or the sample matrix, or due to the gas chromatographic temperature program, a baseline of at least 5 times the width at half-height is permitted.

Symmetry factor (A_S)

The symmetry factor of a peak (also known as the asymmetry factor or tailing factor) (Fig. 2.00-8) is calculated using the following equation:

$$A_S = \frac{w_{0.05}}{2d}$$

$w_{0.05}$: Width of the peak at one-twentieth of the peak height

d: Distance between the perpendicular dropped from the peak maximum and the leading edge of the peak at one-twentieth of the peak height

An A_S value of 1.0 signifies symmetry. When $A_S > 1.0$, the peak is tailing. When $A_S < 1.0$, the peak is fronting.

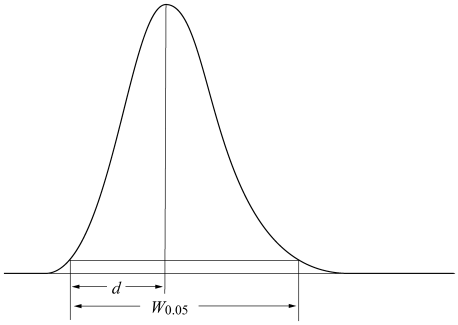


Fig. 2.00-8

System repeatability

The repeatability of response is expressed as an estimated percentage relative standard deviation (%RSD) of a consecutive series of measurements for not fewer than 3 injections or applications of a reference solution, and is calculated using the following equation.

$$\%\text{RSD} = \frac{100}{\bar{y}} \sqrt{\frac{\Sigma (y_i - \bar{y})^2}{n - 1}}$$

y_i: Individual values expressed as peak area, peak height, or ratio of areas by the internal standardization method;
$\bar{y}$: Mean of individual values
n: Number of individual values

Total mobile phase time (t_t)

In size-exclusion chromatography, retention time of a component whose molecules are smaller than the smallest gel pores (Fig. 2.00-5).

Total mobile phase volume (V_t)

In size-exclusion chromatography, retention volume of a component whose molecules are smaller than the smallest gel pores. It may be calculated from the total mobile phase time and the flow rate (F) (mL/minute) using the following equation.

$$V_t = t_t \times F$$

3. System suitability

This section only covers liquid chromatography and gas chromatography.

The various components of the equipment employed must be qualified and be capable of achieving the performance required to conduct the test or assay.

The system suitability tests represent an integral part of the analytical procedure and are used to ensure adequate performance of the chromatographic system. Column plate number, retention factor (mass distribution ratio), system repeatability, signal-to-noise, symmetry factor and resolution/peak-to-valley ratio are the parameters that may be employed in assessing the performance of the chromatographic system. When stated in the individual monograph, in cases of complex chromatographic profiles (e.g., for biotechnological/biological products), visual comparison of the profiles can be used as a system suitability test.

Factors that may affect the chromatographic behavior include:

- Composition and temperature of the mobile phase;
- Ionic strength and pH of the aqueous component of the mobile phase;
- Flow rate, column dimensions, column temperature and pressure;
- Stationary phase characteristics including type of chromatographic support (particle-based or monolithic), particle or pore size, porosity, specific surface area;
- Reversed phase and other surface-modification of the stationary phases, the extent of chemical modification (as expressed by end-capping, carbon loading etc.).

Retention times and relative retentions may be provided in monographs for information purposes only. There are no acceptance criteria applied to relative retentions.

Compliance with the system suitability criteria is required throughout the chromatographic procedure. No sample analysis is acceptable unless the suitability of the system has been demonstrated.

◇When the following criteria are specified in the system suitability tests, each requirement is to be fulfilled unless otherwise prescribed.◇

System repeatability — assay of an active substance or an excipient

In an assay of an active substance or an excipient, where the target value is 100% for a pure substance, and a system repeatability requirement is not specified, the maximum permitted relative standard deviation ($\%\text{RSD}_{max}$) for the defined limits is calculated for a series (n = 3 to 6) of injections of the reference solution.

The maximum permitted relative standard deviation of the peak response does not exceed the appropriate value given in Table 2.00-1.

$$\%\text{RSD}_{max} = \frac{KB\sqrt{n}}{t_{90\%,\, n-1}}$$

K: Constant (0.349), obtained from the expression,
$K = \frac{0.6}{\sqrt{2}} \times \frac{t_{90\%,\,5}}{\sqrt{6}}$ in which $\frac{0.6}{\sqrt{2}}$ represents the required relative standard deviation (percentage) determined on 6 injections for B = 1.0
B: (Upper limit given in the definition of the individual monograph − 100) %
N: Number of replicate injections of the reference solution ($3 \leq n \leq 6$);
$t_{90\%,\,n-1}$: Student's t at the 90% probability level (double sided) with $n - 1$ degrees of freedom.

Table 2.00-1 Maximum permitted relative standarddeviation (assay)

	Number of individual injections *n*			
	3	4	5	6
B (%)	Maximum permitted relative standard deviation (%)			
2.0	0.41	0.59	0.73	0.85
2.5	0.52	0.74	0.92	1.06
3.0	0.62	0.89	1.10	1.27

B: (Upper limit of content given in the individual monograph − 100) %.

System sensitivity

The signal-to-noise ratio is used to define the system sensitivity. The limit of quantitation (corresponding to a signal-to-noise ratio of 10) is equal to or less than the reporting threshold.

Peak symmetry

Unless otherwise stated, in a test or assay, the symmetry factor (tailing factor) of the peak used for quantitation is 0.8 to 1.8.

4. Adjustment of chromatographic conditions

The chromatographic conditions described have been validated during the elaboration of the monograph.

The extent to which the various parameters of a chromatographic test may be adjusted without fundamentally modifying the pharmacopoeial analytical procedures are listed below. Changes other than those indicated require revalidation of the procedure.

Multiple adjustments can have a cumulative effect on the performance of the system and are to be properly evaluated by the users. This is particularly important in cases where the separation pattern is described as a profile. In those cases, a risk assessment has to be carried out.

Any adjustments must be made on the basis of the pharmacopoeial procedure.

If adjustments are made to the procedure, additional verification tests may be required. To verify the suitability of the adjusted pharmacopoeial procedure, assess the relevant analytical performance characteristics potentially affected by the change.

When a pharmacopoeial analytical procedure has been adjusted according to the requirements stated below, no further adjustments are allowed without appropriate revalidation.

Compliance with the system suitability criteria is required to verify that conditions for satisfactory performance of the test or assay are achieved.

Adjustment of conditions with gradient elution (HPLC) or temperature programming (GC) is more critical than with isocratic (HPLC) or isothermal (GC) elution, since it may shift some peaks to a different step of the gradient or to different elution temperatures, potentially causing partial or complete coelution of adjacent peaks or peak inversion, and thus leading to the incorrect assignment of peaks, and to the masking of peaks or a shift such that elution occurs beyond the prescribed elution time.

◇In the tests of biotechnological/biological products such as peptide mapping, glycosylation analysis and tests related to molecular heterogeneity, the separation pattern obtained by liquid chromatography may be set for acceptance criteria as a profile. In such a test method, the method shown in this section may not be applicable.◇

◇Crude drugs and related drugs are outside the scope of this section.◇

4.1. Liquid chromatography: isocratic elution

Column parameters and flow rate

- Stationary phase: No change of the identity of the substituent (e.g. no replacement of C18 by C8); the other physico-chemical characteristics of the stationary phase, i.e. chromatographic support, surface modification and extent of chemical modification must be similar; a change from Totally Porous Particle (TPP) columns to Superficially Porous Particle (SPP) columns is allowed provided the above-mentioned requirements are met.
- Column dimensions (particle size, length): The particle size and/or length of the column may be modified provided that the ratio of the column length (L) to the particle size (d_p) remains constant or in the range between −25% to +50% of the prescribed L/d_p ratio.
- Adjustment from totally porous to superficially porous particles: For the application of particle-size adjustment from totally porous to superficially porous particles, other combinations of L and d_p can be used provided that the plate number (N) is within −25% to +50%, relative to the prescribed column. These changes are acceptable provided system suitability criteria are fulfilled, and selectivity and elution order of the specified impurities to be controlled are demonstrated to be equivalent.
- Internal diameter: In absence of a change in particle size and/or length, the internal diameter of the column may be adjusted.

Caution is necessary when the adjustment results in smaller peak volumes, due to a smaller particle size or a smaller internal diameter, a situation which may require adjustments to minimize extra-column band broadening by factors such as instrument connections, detector cell volume and sampling rate, and injection volume.

When the particle size is changed, the flow rate ◇may require◇ adjustment, because smaller-particle columns will require higher linear velocities for the same performance (as measured by reduced plate height). The flow rate ◇can be adjusted◇ for both the change in column diameter and particle size using the following equation:

$$F_2 = F_1 \times [(d_{c2}^2 \times d_{p1})/(d_{c1}^2 \times d_{p2})]$$

F_1: Flow rate (mL/minute) indicated in the monograph
F_2: Adjusted flow rate (mL/minute)
d_{c1}: Internal diameter (mm) of the column indicated in the monograph

d_{c2}: Internal diameter (mm) of the column used
d_{p1}: Particle size (μm) indicated in the monograph
d_{p2}: Particle size (μm) of the column used

When a change is made from $\geq 3\ \mu$m to $< 3\ \mu$m particles in isocratic separations, an additional increase in linear velocity (by adjusting the flow rate) may be justified, provided that the column performance does not drop by more than 20%. Similarly, when a change is made from $< 3\ \mu$m to $\geq 3\ \mu$m particles, an additional reduction of linear velocity (flow rate) may be justified to avoid reduction in column performance by more than 20%.

After an adjustment due to a change in column dimensions, an additional change in flow rate of ±50% is permitted.

Column temperature: ±10°C, where the operating temperature is specified, unless otherwise prescribed.

Further adjustments in analytical procedure conditions (mobile phase, temperature, pH, etc.) may be required, within the permitted ranges described under System Suitability and Adjustment of chromatographic conditions in this test method.

Mobile phase:

- Composition: The amount of the minor solvent components may be adjusted by ±30% relative. For a minor component at 10% of the mobile phase, a 30% relative adjustment allows a range of 7 – 13%. For a minor component at 5% of the mobile phase, a 30% relative adjustment allows a range of 3.5 – 6.5%. No component is altered by more than 10% absolute. A minor component comprises less than or equal to (100/n) %, *n* being the total number of components of the mobile phase.
- pH of the aqueous component of the mobile phase: ±0.2 pH units, unless otherwise prescribed
- Concentration of salts in the buffer component of a mobile phase: ±10%
- Flow rate: In absence of a change in column dimensions, an adjustment of the flow rate by ±50% is permitted.

Detector wavelength: No adjustment permitted.

Injection volume: When the column dimensions are changed, the following equation may be used for adjusting the injection volume.

$$V_{inj2} = V_{inj1}\ (L_2 d_{c2}^2)/(L_1 d_{c1}^2)$$

V_{inj1}: Injection volume (μL) indicated in the monograph
V_{inj2}: Adjusted injection volume (μL)
L_1: Column length (cm) indicated in the monograph
L_2: New column length (cm)
d_{c1}: Column internal diameter (mm) indicated in the monograph
d_{c2}: New column internal diameter (mm)

This equation may not be applicable to changes from TPP columns to SPP columns.

Even in the absence of any column dimension change, the injection volume may be varied provided System Suitability criteria remain within their established acceptability limits. When the injection volume is decreased, special attention is given to (limit of) detection and repeatability of the peak response(s) to be determined. An increase is permitted provided, in particular, linearity and resolution of the peak(s) to be determined remain satisfactory.

4.2. Liquid chromatography: gradient elution

Adjustment of chromatographic conditions for gradient systems requires greater caution than for isocratic systems.

Column parameters and flow rate

- Stationary phase: No change of the identity of the substituent (e.g. no replacement of C18 by C8). The other physico-chemical characteristics of the stationary phase, i.e. chromatographic support; surface modification and extent of chemical modification must be similar. A change from Totally Porous Particle (TPP) columns to Superficially Porous Particle (SPP) columns is allowed provided the above-mentioned requirements are met.
- Column dimensions (particle size, length): The particle size and/or length of the column may be modified provided that the ratio of the column length (L) to the particle size (d_p) remains constant or in the range between −25% to +50% of the prescribed L/d_p ratio.
  Adjustments from totally porous to superficially porous particles: For the application of particle-size adjustment from totally porous to superficially porous particles, other combinations of L and d_p can be used provided that the ratio $(t_R/w_h)^2$ is within −25% to +50%, relative to the prescribed column, for each peak used to check the system suitability, as stated in this chapter and the individual monograph.
  These changes are acceptable provided system suitability criteria are fulfilled, and selectivity and elution order of the specified impurities to be controlled are demonstrated to be equivalent.
- Internal diameter: In absence of a change in particle size and/or length, the internal diameter of the column may be adjusted.

Caution is necessary when the adjustment results in smaller peak volumes, due to a smaller particle size or a smaller internal diameter, a situation which may require adjustments to minimize extra-column band broadening by factors such as instrument connections, detector cell volume and sampling rate, and injection volume.

When the particle size is changed, the flow rate ◇may require◇ adjustment, because smaller-particle columns will require higher linear velocities for the same performance (as measured by reduced plate height). The flow rate ◇can be adjusted◇ for both the change in column diameter and particle size using the following equation:

$$F_2 = F_1 \times [(d_{c2}^2 \times d_{p1})/(d_{c1}^2 \times d_{p2})]$$

F_1: Flow rate (mL/minute) indicated in the monograph
F_2: Adjusted flow rate (mL/minute)
d_{c1}: Internal diameter (mm) of the column indicated in the monograph
d_{c2}: Internal diameter (mm) of the column used
d_{p1}: Particle size (μm) indicated in the monograph

d_{p2}: Particle size (μm) of the column used

A change in column dimensions, and thus in column volume, impacts the gradient volume which controls selectivity. Gradients are adjusted to the column volume by changing the gradient volume in proportion to the column volume. This applies to every gradient segment volume. Since the gradient volume is the gradient time, t_G, multiplied by the flow rate, F, the gradient time for each gradient segment needs to be adjusted to maintain a constant ratio of the gradient volume to the column volume (expressed as $L \times d_c^2$). Thus, the new gradient time, t_{G2} can be calculated from the original gradient time, t_{G1}, the flow rate(s), and the column dimensions as follows.

$$t_{G2} = t_{G1} \times (F_1/F_2)\ [(L_2 \times d_{c2}^2)/(L_1 \times d_{c1}^2)]$$

Thus, the change in conditions for gradient elution requires three steps:

(1) adjust the column length and particle size according to L/d_p,
(2) adjust the flow rate for changes in particle size and column diameter, and
(3) adjust the gradient time of each segment for changes in column length, diameter and flow rate. The example below illustrates this process.

Variable	Original Conditions	Adjusted Conditions	Comment
Column length (L) (mm)	150	100	User's choice
Column diameter (d_c) (mm)	4.6	2.1	User's choice
Particle size (d_p) (μm)	5	3	User's choice
L/d_p	30.0	33.3	(1)
Flow rate (mL/min)	2.0	0.7	(2)
Gradient adjustment factor (t_{G2}/t_{G1})		0.4	(3)
Gradient conditions			
B (%)	Time (min)	Time (min)	
30	0	0	
30	3	(3 × 0.4) = 1.2	
70	13	[1.2 + (10 × 0.4)] = 5.2	
30	16	[5.2 + (3 × 0.4)] = 6.4	

(1) 11% increase within allowed L/d_p change of − 25% to + 50%

(2) calculated using $F_2 = F_1\ [(d_{c2}^2 \times d_{p1})/(d_{c1}^2 \times d_{p2})]$
(3) calculated using $t_{G2} = t_{G1} \times (F_1/F_2)\ [(L_2 \times d_{c2}^2)/(L_1 \times d_{c1}^2)]$

- Column temperature: ±5°C, where the operating temperature is specified, unless otherwise prescribed.

Further adjustments in analytical procedure conditions (mobile phase, temperature, pH, etc.) may be required, within the permitted ranges described under System Suitability and Adjustment of Chromatographic Conditions in this chapter.

Mobile phase

- Composition/gradient: Adjustments of the composition of the mobile phase and the gradient are acceptable provided that.
  (i) The system suitability criteria are fulfilled.
  (ii) The principal peak(s) elute(s) within ±15% of the retention time(s) obtained with the original conditions. This requirement does not apply when the column dimensions are changed.
  (iii) The composition of the mobile phase and the gradient are such that the first peaks are sufficiently retained and the last peaks are eluted.
- pH of the aqueous component of the mobile phase: ±0.2 pH units, unless otherwise prescribed.
- Concentration of salts in the buffer component of a mobile phase: ±10%

Where compliance with the system suitability criteria cannot be achieved, it is preferable to consider the dwell volume or to change the column.

Dwell volume The configuration of the equipment employed may significantly alter the resolution, retention time and relative retentions described. Should this occur, it may be due to a change in dwell volume. Monographs preferably include an isocratic step before the start of the gradient program so that an adaptation can be made to the gradient time points to take account of differences in dwell volume between the system used for analytical procedure development and that actually used. It is the user's responsibility to adapt the length of the isocratic step to the analytical equipment used. If the dwell volume used during the elaboration of the monograph is given in the monograph, the time points (t min) stated in the gradient table may be replaced by adapted time points (t_c min), calculated using the following equation.

$$t_c = t - (D - D_0)/F$$

D: Dwell volume (mL)
D_0: Dwell volume (mL) used for development of the analytical procedure
F: Flow rate (mL/min)

The isocratic step introduced for this purpose may be omitted if validation data for application of the analytical procedure without this step is available.

Detector wavelength: No adjustment permitted.

Injection volume: When the column dimensions are changed, the following equation may be used for adjusting the injection volume.

$$V_{inj2} = V_{inj1} (L_2 d_{c2}^2)/(L_1 d_{c1}^2)$$

V_{inj1}: Injection volume (μL) indicated in the monograph
V_{inj2}: Adjusted injection volume (μL)
L_1: Column length (cm) indicated in the monograph
L_2: New column length (cm)
d_{c1}: Column internal diameter (mm) indicated in the monograph
d_{c2}: New column internal diameter (mm)

This equation may not be applicable to changes from TPP columns to SPP columns.

Even in the absence of any column dimension change, the injection volume may be varied provided system suitability criteria remain within their established acceptability limits. When the injection volume is decreased, special attention is given to (limit of) detection and repeatability of the peak response(s) to be determined. An increase is permitted provided, in particular, linearity and resolution of the peak(s) to be determined remain satisfactory.

4.3. Gas chromatography

Column parameters

Stationary phase:

Particle size: Maximum reduction of 50%. No increase permitted (packed columns).

Film thickness: −50% to +100% (capillary columns)

Column dimensions:

Length: −70% to +100%;

Internal diameter: ±50%;

Column temperature: ±10%;

Temperature program: Adjustment of temperatures is permitted as stated above. Adjustment of ramp rates and hold times of up to ±20% is permitted.

Flow rate: ±50%.

The above changes are acceptable provided system suitability criteria are fulfilled, and selectivity and elution order of the specified impurities to be controlled are demonstrated to be equivalent.

Injection volume and split ratio: may be varied provided system suitability criteria remain within their established acceptability limits. When the injection volume is decreased, or the split ratio is increased, special attention is given to (limit of) detection and repeatability of the peak response(s) to be determined. An increase in injection volume or a decrease in split ratio is permitted provided, in particular, linearity and resolution of the peak(s) to be determined remain satisfactory.

Injection port temperature and transfer-line temperature in static head-space conditions: ±10°C, provided no decomposition or condensation occurs.

5. Quantitation

The following quantitation approaches may be used in general tests or monographs.

5.1. External standard method

Using a calibration function

Standard solutions with several graded amounts of a reference standard of the compound to be analyzed are prepared in a range that has been demonstrated to give a linear response, and a fixed volume of these standard solutions is injected. With the chromatograms obtained, a calibration function is prepared by plotting the peak areas or peak heights on the ordinate against the amount of reference standard on the abscissa. The calibration function is generally obtained by linear regression. Then, a sample solution is prepared according to the procedure specified in the individual monograph. The chromatography is performed under the same operating conditions as for the preparation of the calibration function, the peak area or peak height of the compound to be analyzed is measured, and the amount of the compound is read out or calculated from the calibration function.

Using one-point calibration

In an individual monograph, generally one of the standard solutions with a concentration within the linear range of the calibration function and a sample solution with a concentration close to that of the standard solution are prepared, and the chromatography is performed under fixed conditions to obtain the amount of the component by comparing the responses obtained. In this method, all procedures, such as the injection, must be carried out under constant conditions.

5.2. Internal standard method

Using a calibration function

In the internal standard method, a stable compound is chosen as an internal standard which shows a retention time close to that of the compound to be analyzed, and whose peak is well separated from all other peaks in the chromatogram.

Several standard solutions containing a fixed amount of the internal standard and graded amounts of a reference standard of the compound to be analyzed are prepared. Based on the chromatograms obtained by injection of a fixed volume of individual standard solutions, the ratio of peak area or peak height of the reference standard to that of the internal standard is calculated. A calibration function by plotting these ratios on the ordinate against the amount of the reference standard or the ratio of the amount of reference standard to that of the internal standard on the abscissa is prepared. The calibration function is generally obtained by linear regression.

Then, a sample solution containing the internal standard in the same amount as in the standard solutions used for the preparation of the calibration function is prepared according to the procedure specified in the individual monograph. The chromatography is performed under the same operating conditions as for the preparation of the calibration function. The ratio of the peak area or peak height of the compound to be analyzed to that of the internal standard is calculated, and the amount of the compound is read out or calculated from the calibration function.

Using one point calibration

In an individual monograph, generally one of the standard solutions with a concentration within the linear range of the calibration function and a sample solution with a concentration close to that of the standard solution, both containing a fixed amount of the internal standard, are prepared, and the chromatography is performed under fixed conditions to determine the amount of the compound to be analyzed by comparing the ratios obtained.

5.3 Normalisation procedure

Provided linearity of the peaks has been demonstrated, individual monographs may prescribe that the percentage content of a component of the substance to be examined is calculated by determining the area of the corresponding peak as a percentage of the total area of all the peaks, excluding those due to solvents or reagents or arising from the mobile phase or the sample matrix, and those at or below the disregard limit or reporting threshold.

6. Other considerations

6.1. Detector response

The detector sensitivity is the signal output per unit concentration or unit mass of a substance in the mobile phase entering the detector. The relative detector response factor, commonly referred to as response factor, expresses the sensitivity of a detector for a given substance relative to a standard substance. The correction factor is the reciprocal of the response factor. In tests for related substances any correction factors indicated in the monograph are applied (i.e. when the response factor is outside the range 0.8-1.2).

6.2. Interfering peaks

Peaks due to solvents and reagents or arising from the mobile phase or the sample matrix are disregarded.

6.3. Measurement of peaks

Integration of the peak area of any impurity that is not completely separated from the principal peak is preferably performed by tangential skim (Fig. 2.00-9).

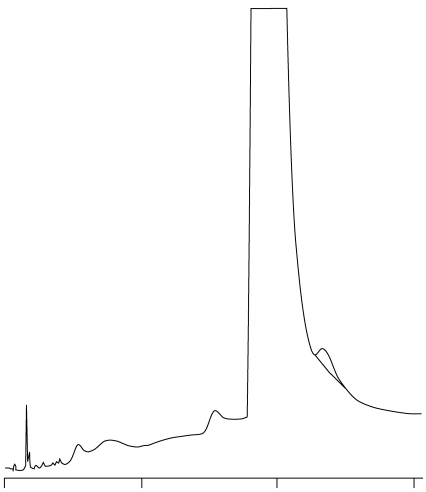


Fig. 2.00-9

6.4. Reporting threshold

When the related substances test prescribes a limit for the total of impurities or a quantitative determination of an impurity, it is important to choose an appropriate reporting threshold and appropriate conditions for the integration of the peak areas. In such tests the reporting threshold, i.e. the limit above which a peak is reported, is generally 0.05%.

Change the following as follows:

2.01 Liquid Chromatography

Liquid Chromatography is a method to develop a mixture injected into a column prepared with a suitable stationary phase by passing a liquid as a mobile phase through the column, in order to separate the mixture into its components by making use of the difference of retention capacity against the stationary phase, and to determine the components. This method can be applied to a liquid or soluble sample, and is used for identification, purity test, and quantitative determination.

1. Apparatus

Basically, the apparatus required for the liquid chromatographic procedure consists of a pumping system for the mobile phase, a sample injection port, a column, a detector and a recorder. A mobile phase component regulator, a thermostat for the column, a pumping system for reaction reagents and a chemical reaction chamber are also used, if necessary. The pumping system serves to deliver the mobile phase and the reagents into the column and connecting tube at a constant flow rate. The sample injection port is used to deliver a quantity of the sample to the apparatus with high reproducibility. The column is a tube with a smooth interior, made of inert metal, etc., in which a packing material for liquid chromatography is uniformly packed. A column with a stationary phase chemically bound on the inside wall instead of the column packed with the packing material may be used. The detector is used to detect a property of the samples which is different from that of the mobile phase, and may be an ultraviolet or visible spectrophotometer, fluorometric detector, differential refractometer, electrochemical detector, chemiluminescence detector, electric conductivity detector, mass spectrophotometer, etc. The output signal is usually proportional to the concentration of samples at amounts of less than a few μg. The recorder is used to record the output signals of the detector. As required, a data processor may be used as the recorder to record or output the chromatogram, retention times or amounts of the components. The mobile phase component regulator is used to vary the ratio of the mobile phase components in a stepwise or gradient fashion.

2. Procedure

Fix the detector, column and mobile phase to the apparatus, and adjust the flow rate and the column temperature to the values described in the operating conditions specified in the individual monograph. Inject a volume of the sample solution or the standard solution specified in the individual monograph with the sample injector into the column

through the sample injection port. The separated components are detected by the detector, and recorded by the recorder as a chromatogram. If the components to be analyzed have no readily detectable physical properties such as absorbance or fluorescence, the detection is achieved by changing the components to suitable derivatives. Usually, the derivatization is performed as a pre- or post-column labeling.

3. Identification and purity test

When Liquid Chromatography is used for identification of a component of a sample, it is performed by confirming identity of the retention time of the component and that of an authentic specimen, or by confirming that the peak shape of the component is unchanged after mixing the sample with an authentic specimen. If a detector which is able to obtain chemical structural information of the component at the same time is used, highly specific identification can be achieved by confirming identity of the chemical structure of the component and that of an authentic specimen, in addition to the identity of their retention times.

When Liquid Chromatography is used for purity test, it is generally performed by comparing the peak area of target impurity from the sample solution with that of the main component from a standard solution, which is prepared by diluting the sample solution to a concentration corresponding to the specified limit of the impurity, or by calculating target impurity content using the peak area percentage method. Unless otherwise specified, if a sample is separated into isomers in the chromatogram, the isomer ratio is calculated by using the peak area percentage method.

The peak area percentage method is a method to calculate the proportion of the components from the ratio of the peak area of each component to the sum of the peak areas of every peak recorded in the chromatogram. In order to obtain accurate results in evaluating the proportion of the components, it is necessary to correct the area of each component based on its correction factor to the principal component.

4. Assay

4.1. Internal standard method

In the internal standard method, choose a stable compound as an internal standard which shows a retention time close to that of the compound to be assayed, and whose peak is well separated from all other peaks in the chromatogram. Prepare several kinds of standard solutions containing a fixed amount of the internal standard and several graded amounts of the authentic specimen specified in the individual monograph. Based on the chromatogram obtained by injection of a fixed volume of individual standard solutions, calculate the ratio of peak area or peak height of the authentic specimen to that of the internal standard, and prepare a calibration curve by plotting these ratios on the ordinate against the amount of the authentic specimen or the ratio of the amount of the authentic specimen to that of the internal standard on the abscissa. The calibration curve is usually obtained as a straight line passing through the origin. Then, prepare a sample solution containing the internal standard in the same amount as in the standard solutions used for the preparation of the calibration curve according to the method specified in the individual monograph, perform the liquid chromatography under the same operating conditions as for the preparation of the calibration curve, calculate the ratio of the peak area or peak height of the objective compound to that of the internal standard, and read the amount of the compound from the calibration curve.

In an individual monograph, generally one of the standard solutions with a concentration within the linear range of the calibration curve and a sample solution with a concentration close to that of the standard solution are prepared, and the chromatography is performed with these solutions under fixed conditions to determine the amount of the objective compound.

4.2. Absolute calibration curve method

Prepare standard solutions with several graded amounts of the authentic specimen, and inject accurately a fixed volume of these standard solutions. With the chromatogram obtained, prepare a calibration curve by plotting the peak areas or peak heights on the ordinate against the amount of the authentic specimen on the abscissa. The calibration curve is generally obtained as a straight line passing through the origin. Then, prepare a sample solution according to the method specified in the individual monograph, perform the liquid chromatography under the same conditions as for the preparation of the calibration curve, measure the peak area or peak height of the objective compound, and read the amount of the compound from the calibration curve.

In an individual monograph, generally one of the standard solutions with a concentration within the linear range of the calibration curve and a sample solution with a concentration close to that of the standard solution are prepared, and the chromatography is performed with these solutions under a fixed condition to obtain the amount of the component. In this method, all procedures, such as the injection procedure, must be carried out under a strictly constant condition.

5. Method for peak measuring

Generally, the following methods are used.

5.1. Peak height measuring method

(i) Peak height method: Measure the distance between the maximum of the peak and the intersecting point of a perpendicular line from the maximum of the peak to the horizontal axis of recording paper with a tangent linking the baselines on both sides of the peak.

(ii) Automatic peak height method: Measure the signals from the detector as the peak height using a data processing system.

5.2. Peak area measuring method

(iii) Width at half-height method: Multiply the peak width at the half-height by the peak height.

(iv) Automatic integration method: Measure the signals from the detector as the peak area using a data processing system.

6. System suitability

System suitability testing is an integral part of test methods using chromatography, and is used to ensure that the performance of the chromatographic systems used is as suitable for the analysis of the drug as was at the time when the verification of the test method was performed using the system. System suitability testing should be carried out at every series of drug analysis. The test procedures and acceptance criteria of system suitability testing must be prescribed in the test method of drugs. The results of drug analyses are not acceptable unless the requirements of system suitability have been met.

In system suitability testing of the chromatographic systems, the evaluation of ''System performance'' and ''System repeatability'' is usually required. For quantitative purity tests, the evaluation of ''Test for required detectability'' may also be required. If appropriate, system suitability can also be evaluated by the parameters of system suitability prescribed in Chromatography <*2.00*>. However, Liquid Chromatography <*2.01*> and Chromatography <*2.00*> cannot be applied in combination.

6.1. Test for required detectability

For purity tests, when it is confirmed that the target impurity is distinctly detected at the concentration of its specification limit, it is considered verified that the system used has adequate performance to achieve its intended use.

For quantitative purity tests, ''Test for required detectability'' is usually required, and in order to confirm, in some degree, the linearity of response near its specification limit, the range of expected response to the injection of a certain volume of target impurity solution at the concentration of its specification limit should be prescribed. For limit test, ''Test for required detectability'' is not required, if the test is performed by comparing the response from sample solution with that from standard solution at the concentration of its specification limit. ''Test for required detectability'' is also not required, if it is confirmed that the impurity can be detected at its specification limit by the evaluation of ''System repeatability'' or some other procedure.

6.2. System performance

When it is confirmed that the specificity for determining the test ingredient is ensured, it is considered verified that the system used has adequate performance to achieve its intended use.

In assay, ''System performance'' should be defined by the resolution between the test ingredient and a target substance to be separated (a closely eluting compound is preferable), and when appropriate, by their order of elution. In purity tests, both the resolution and the order of elution between the test ingredient and a target substance to be separated (a closely eluting compound is preferable) should be prescribed. In addition, if necessary, the symmetry factor of the test ingredient should be prescribed together with them. However, if there is no suitable target substance to be separated, it is acceptable to define ''System performance'' using the number of theoretical plates and the symmetry factor of the test ingredient.

6.3. System repeatability

When it is confirmed that the degree of variation (precision) of the response of the test ingredient is at a level that meets the requirement of ''System repeatability'', it is considered verified that the system used has adequate performance to achieve its intended use.

The allowable limit of ''System repeatability'' is normally defined as the relative standard deviation (*RSD*) of the response of the test ingredient in replicate injections of standard solution. It is acceptable to confirm the repeatability of the system not only by replicate injections of standard solution before sample injections, but also by divided injections of standard solution before and after sample injections, or by interspersed injections of standard solution among sample injections.

In principle, total number of replicate injections should be 6. However, in the case that a long time is necessary for one analysis, such as the analysis using the gradient method, or the analysis of samples containing late eluting components, it may be acceptable to decrease the number of replicate injections by adopting new allowable limit of ''System repeatability'' which can guarantee a level of ''System repeatability'' equivalent to that at 6 replicate injections.

The allowable limit of ''System repeatability'' should be set at an appropriate level based on the data when suitability of the method for the evaluation of quality of the drug was verified, and the precision necessary for the quality test.

7. Point to consider on changing the operating conditions

Among the operating conditions specified in the individual monograph, inside diameter and length of the column, particle size of the packing material (pore size in the case of monolithic columns), column temperature, composition ratio of the mobile phase, composition of buffer solutions in the mobile phase, pH of the mobile phase, concentration of ion-pair forming agents in the mobile phase, ionic strength of the mobile phase, gradient program, flow rate of the mobile phase, number and timing of mobile phase composition changes in gradient program, flow rate of mobile phase in gradient program, composition and flow rate of derivatizing reagents, and reaction time and chamber temperature in chemical reaction may be partially modified after the analytical performance is appropriately verified. However, for crude drugs etc., conformance to the specifications of the system suitability may be substituted for the verification of analytical performance.

8. Terminology The terminology used conforms to the definition in Chromatography <*2.00*>.

9. Note

Avoid the use of authentic specimens, internal standards, reagents or solvents containing substances that may interfere with the determination.

Change the following as follows:

2.02 Gas Chromatography

Gas Chromatography is a method to develop a mixture injected into a column prepared with a suitable stationary phase by passing a gas (carrier gas) as a mobile phase through the column, in order to separate the mixture into its components by making use of the difference of retention capacity against the stationary phase, and to determine the components. This method can be applied to a gaseous or vaporizable sample, and is used for identification, purity test, and quantitative determination.

1. Apparatus

Basically, the apparatus required for the gas chromatographic procedure consists of a carrier gas-introducing port and flow regulator, a sample injection port, a column, a column oven, a detector and a recorder. A gas-introducing port and flow regulator for a combustion gas, a burning supporting gas and an accessory gas and sample injection port for headspace are also used, if necessary. The carrier gas-introducing port and flow regulator serves to deliver the carrier gas into the column at a constant flow rate, and usually consist of a pressure regulation valve, a flow rate regulation valve and a pressure gauge. The sample injection port is used to deliver a quantity of the sample to the flow line of carrier gas with high reproducibility. There are sample injection ports for packed column and for capillary column. There are both divided injection mode and non-divided injection mode to sample injection port for capillary column. The columns are usually classified as packed column or capillary column. The packed column is a tube made of inert metal, glass or synthetic resin, in which a packing material for gas chromatography is uniformly packed. The packed column with not more than 1 mm in inside diameter is also called a packed capillary column (micro packed column). A capillary column is a tube made of inert metal, glass, quartz or synthetic resin, whose inside wall is bound chemically with stationary phase for gas chromatography. The column oven has the setting capacity for a column with required length and the temperature regulation system for keeping the constant column temperature. The detector is used to detect a component separated on the column, and may be an alkaline thermal ionization detector, a flame photometry detector, mass spectrophotometer, hydrogen flame-ionization detector, an electron capture detector, a thermal conductivity detector, etc. The recorder is used to record the output signals of the detector.

2. Procedure

Unless otherwise specified, proceed by the following method. Fix the detector, column and carrier gas to the apparatus, and adjust the flow rate and the column temperature to the values described in the operating conditions specified in the individual monograph. Inject a volume of the sample solution or the standard solution specified in the individual monograph with the sample injector into the column system through the sample injection port. The separated components are detected by the detector, and recorded by the recorder as a chromatogram.

3. Identification and purity test

Identification of a component of a sample is performed by confirming identity of the retention time of the component and that of an authentic specimen, or by confirming that the peak shape of the component is unchanged after mixing the sample with an authentic specimen.

In general, the purity of the sample is determined by comparing the peak area of target impurity from the sample solution with that of the main component from a standard solution, which is prepared by diluting the sample solution to a concentration corresponding to the specified limit of the impurity, or by calculating target impurity content using the peak area percentage method. Unless otherwise specified, if a sample is separated into isomers in the chromatogram, the isomer ratio is calculated by using the peak area percentage method.

The peak area percentage method is a method to calculate the proportion of the components from the ratio of the peak area of each component to the sum of the peak areas of every peak recorded in the chromatogram. In order to obtain accurate results in evaluating the proportion of the components, it is necessary to correct the area of each component based on its response factor to the principal component.

4. Assay

In general, perform the assay by using the internal standard method. The absolute calibration curve method is used when a suitable internal standard is not available. Perform the assay by using the standard addition method when the effect of the component other than the compound to be assayed on the quantitative determination is not negligible against a result of the determination.

4.1 Internal standard method

In the internal standard method, choose a stable compound as an internal standard which shows a retention time close to that of the compound to be assayed, and whose peak is well separated from all other peaks in the chromatogram. Prepare several kinds of standard solutions containing a fixed amount of the internal standard and several graded amounts of the authentic specimen specified in the individual monograph. Based on the chromatogram obtained by injection of a fixed volume of individual standard solutions, calculate the ratio of peak area or peak height of the authentic specimen to that of the internal standard, and prepare a calibration curve by plotting these ratios on the ordinate against the amount of the authentic specimen or the ratio of the amount of the authentic specimen to that of the internal standard on the abscissa. The calibration curve is usually obtained as a straight line passing through the origin. Then, prepare a sample solution containing the internal standard in the same amount as in the standard solutions used for the preparation of the calibration curve according to the method specified in the individual monograph, perform the gas chromatography under the same operating conditions as for the preparation of the calibration curve,

calculate the ratio of the peak area or peak height of the objective compound to that of the internal standard, and read the amount of the compound from the calibration curve.

In an individual monograph, generally one of the standard solutions with a concentration within the linear range of the calibration curve and a sample solution with a concentration close to that of the standard solution are prepared, and the chromatography is performed with these solutions under fixed conditions to determine the amount of the objective compound.

4.2 Absolute calibration curve method

Prepare standard solutions with several graded amounts of the authentic specimen, and inject accurately a fixed volume of these standard solutions. With the chromatogram obtained, prepare a calibration curve by plotting the peak areas or peak heights on the ordinate against the amount of the authentic specimen on the abscissa. The calibration curve is generally obtained as a straight line passing through the origin. Then, prepare a sample solution according to the method specified in the individual monograph, perform the gas chromatography under the same conditions as for the preparation of the calibration curve, measure the peak area or peak height of the objective compound, and read the amount of the compound from the calibration curve.

In an individual monograph, generally one of the standard solutions with a concentration within the linear range of the calibration curve and a sample solution with a concentration close to that of the standard solution are prepared, and the chromatography is performed with these solutions under a fixed condition to obtain the amount of the component. In this method, all procedures, such as the injection procedure, must be carried out under a strictly constant condition.

4.3 Standard addition method

Pipet a fixed volume of more than 4 sample solutions, add exactly the standard solution so that stepwise increasing amounts of the object compound are contained in the solutions except 1 sample solution, diluted exactly each solution with and without standard solution to a definite volume, and use each solution as the sample solution. Based on the chromatogram obtained by exact injection of a fixed volume of individual sample solutions, measure the peak area or peak height of individual sample solutions. Calculate the concentration of standard objective compound added into each sample solution, plot the amounts (concentration) of added standard object compound on the abscissa and the peak area or peak height on the ordinate on the graph, extend the calibration curve obtained by linking the plots, and determine the amount of object compound to be assayed from the distance between the origin and the intersecting point of the calibration curve with the abscissa. This method is available only in the case that the calibration curve is a straight line, and passes through the origin when the absolute calibration curve method is employed. In this method, all procedures must be carried out under a strictly constant condition.

5. Method for peak measuring

Generally, the following methods are used.

5.1 Peak height measuring method

(i) Peak height method: Measure the distance between the maximum of the peak and the intersecting point of a perpendicular line from the maximum of the peak to the horizontal axis of recording paper with a tangent linking the baselines on either side of the peak.

(ii) Automatic peak height method: Measure the signals from the detector as the peak height using a data processing system.

5.2 Peak area measuring method

(i) Width at half-height method: Multiply the peak width at the half-height by the peak height.

(ii) Automatic integration method: Measure the signals from the detector as the peak area using a data processing system.

6. System suitability

Refer to ''System suitability'' described under Liquid Chromatography <*2.01*>.

7. Point to consider in changing the operating conditions

Among the operating conditions specified in the individual monograph, inside diameter and length of column, particle size of packing material, concentration or thickness of stationary phase, column temperature, temperature-rising rate, kind and flow rate of carrier gas, and split ratio may be partially modified after the analytical performance is appropriately verified. However, for crude drugs etc., conformance to the specification of the system suitability may be substituted for the verification of analytical performance. Headspace sample injection device and its operating conditions may be also modified, provided that they give equivalent or more accuracy and precision.

8. Terminology

The terminology used conforms to the definition in Chromatography <*2.00*>.

9. Note

Avoid the use of authentic specimens, internal standards, reagents or solvents containing substances that may interfere with the determination.

Change the following as follows:

2.22 Fluorometry

Fluorometry is a method to measure the intensity of fluorescence emitted from a solution of fluorescent substance irradiated with an exciting light in a certain wavelength range. This method is also applied to the phosphorescent substances.

Fluorescence intensity F in a dilute solution is proportional to the concentration c in mol per liter of the solution and the path length l of light through the solution in centimeter.

$$F = kI_0\phi\varepsilon cl$$

k: Constant
I_0: Intensity of exciting light
ϕ: Quantum yield of fluorescence or phosphorescence

Quantum yield of fluorescence or phosphorescence
= number of quanta as fluorescence or phosphorescence/number of quanta absorbed

ε: Molar extinction coefficient of the substance at the excitation wavelength

1. Apparatus

Spectrofluorometer is usually used. Generally, a xenon-lamp, laser, an alkaline halide lamp, etc. which provide stable exciting light are used as the light source. Usually, a nonfluorescent quartz cell (1 cm × 1 cm) with four transparent sides is used as the container for sample solution.

2. Procedure

Excitation spectrum is obtained by measuring fluorescence intensities of sample solution with varying excitation wavelengths at a fixed emission wavelength and drawing a curve showing the relationship between the excitation wavelength and the fluorescence intensity. Fluorescence spectrum is obtained by measuring fluorescence intensities of sample solution with varying emission wavelengths at a fixed excitation wavelength and drawing the same curve as described for the excitation spectrum. If necessary, the spectra are corrected with regard to the optical characteristics of the apparatus.

The fluorescence intensity is usually measured at the excitation and the emission wavelengths in the vicinity of excitation and emission maxima of the fluorescent substance. The fluorescence intensity is expressed as a value relative to that of a standard solution, because it is readily affected even by a slight change in the condition for the measurement.

Unless otherwise specified, the instrument is operated as follows with standard, sample, and reference solutions prepared as directed in the monograph: Fix the excitation and fluorescence wavelength scales at the designated positions, adjust the dark current to zero, put the quartz cell containing the standard solution in the light path, and adjust the instrument so that the standard solution shows the fluorescence intensity of 60% to 80% of full scale. Then perform the measurements with the cells containing the sample solution and the control solution, and read the fluorescence intensity as % under the same condition. Set the width of the wavelength properly unless otherwise specified.

3. Note

The fluorescence intensity is readily affected by the concentration, temperature and pH of the solution, and nature and purity of solvents or reagents used.

Add the following:

2.27 Near Infrared Spectrometry

Near infrared spectrometry is one of spectroscopic methods used to qualitatively and quantitatively evaluate samples from the analysis of data obtained by determining their absorption spectrum of light in the near-infrared range.

The near-infrared range lies between the visible light and infrared light, typically of wavelengths (or wave numbers) between 750 and 2500 nm (13,333 to 4000 cm^{-1}). The absorption of near-infrared light occurs due to harmonic overtones from normal vibration or combination tones in the infrared range from 2500 to 25000 nm (4000 to 400 cm^{-1}), primarily absorption of O-H, N-H, C-H and S-H that involve hydrogen atoms, in particular.

Absorption in the near-infrared range is far weaker than absorption due to normal vibration that occurs in the infrared range. Furthermore, in comparison with visible light, near-infrared light has longer wavelength, which makes it possible for the light to penetrate to a depth of several mm into solid samples including fine particles. This method is often utilized as a nondestructive analysis, as changes occurring with an absorbed light spectrum (transmitted light or reflected light) in this process provide physical and chemical information pertaining to samples.

Near-infrared spectrometry is used as a rapid and nondestructive method of analysis that replaces conventional and established analysis methods. It is necessary to perform a comparison test to evaluate this method against an existing analysis method, to verify that this method is equivalent to such existing analysis method, before using this analysis method as an evaluation test method for quality control.

Applications of this method include qualitative or quantitative evaluation of active ingredients, additives or water contents of drug substances or preparations. Furthermore, near-infrared spectrometry can also be used for the evaluation of physical conditions of substances, such as crystal forms, crystallinity, particle diameters. It is also possible to perform spectrometry on samples that are located in a remote location away from equipment main units, without sampling, by using optical fibers. It can therefore be used as an effective means to perform pharmaceutical manufacturing process control online (or in-line).

1. Equipment

Near-infrared spectrophotometers mainly include a distributed near-infrared spectrophotometer and a Fourier transform near-infrared spectrophotometer.

1.1. Distributed near-infrared spectrophotometer

This equipment is comprised of a light source section, sample section, spectrometry section, photometry section, signal processing section, data processing section, and display-record output section. Halogen lamps, tungsten lamps, light emitting diodes and other such devices that can emit high intensity near-infrared light in a stable manner are used in the light source section. The sample section is comprised

of a sample cell and a sample holder. Equipment with an optical fiber section that is comprised of optical fibers and a collimator is equipped with a function for transmitting light to the sample section, which is remotely located away from the spectrophotometer main unit. Quartz is ordinarily used as material for optical fibers.

The spectrometry section is intended to extract light of required wavelength, using dispersive devices and is comprised of slits, mirrors and dispersive devices. The photometry section is comprised of detectors and amplifiers. Sensors include semiconductor detectors, as well as photomultiplier tubes. Detecting methods that use semiconductor detectors generally perform detections with single elements, but there are also occasions where array type detectors that use multiple elements are used. Such detectors are capable of simultaneously detecting multiple wavelengths (or wave numbers). The signal processing section separates signals required for measurements from output signals fed by amplifiers and then outputs such isolated signals. The signal processing methods include analog processing and digital processing.

1.2. Fourier transform near-infrared spectrophotometer

The configuration of the equipment is fundamentally the same as that of the distributed-type equipment described in Section 1.1., except for the spectrophotometry section and the signal processing section.

The spectrophotometry section is comprised of interferometers, sampling signal generators, detectors, amplifiers, A/D conversion devices, etc. The signal processing section is equipped with functions that are required for the spectrometer, as well as a function for translating an acquired interference waveform (interferogram) into an absorption spectrum by Fourier transformation.

2. Measurement method

There are three types of measurement methods that are used with near-infrared spectrometry: transmittance method, diffuse reflectance method and transmittance reflectance method. The selection of measurement methods relies on the shape of samples and applications. For example, the transmittance method or diffuse reflectance method is used for solid samples, including fine particles, and the transmittance method or transmittance reflectance method is used for liquid samples. The measurement mode, etc. of equipment are selected and set.

2.1. Transmittance method

The degree of decay for incident light intensity as the light from a light source passes through a sample, is represented as transmittance rate T (%) or absorbance A with the transmittance method.

This method is applied for taking measurements of samples that are liquids and solutions. Quartz glass cells and flow cells are used, with the layer length of 1 – 5 mm along. Furthermore, this method can also be applied for taking measurements of samples that are solids including fine particles. It is also known as the diffuse transmittance method. Selecting appropriate layer length is critical for this method, since the transmitted light intensity varies depending on grain sizes and surface condition of samples.

2.2. Diffuse reflectance method

The ratio of reflection light intensity I emitted from a sample in a wide reflectance range and control reflection light intensity I_r emitted from the surface of a control substance is expressed as reflectance R (%) with the diffuse reflectance method. The near-infrared light penetrates to a depth of several mm into solid samples including fine particles. In that process, transmission, refraction, reflection and dispersion are repeated, and diffusion takes place, but a portion of the diffused light is emitted again from the surface of the sample and captured by a detector. The spectrum for the diffuse reflectance absorbance (A_r) can ordinarily be obtained by plotting logarithm of inverse numbers for reflectance against wavelengths (or wave numbers).

This method is applied to solid samples including fine particles, and requires a diffuse reflector such as a probe.

2.3. Transmittance reflectance method

The transmittance reflectance method is a combination of the transmittance method and reflectance method. A mirror is used to re-reflect the light that has passed through a sample in order to measure transmittance reflectance rate, T^* (%). The light path must be twice the thickness of the sample. On the other hand, the light reflected by a mirror and being introduced into a detector is used as the control light. When this method is applied to suspended samples, however, a metal plate or a ceramic reflector with a rough surface that causes diffuse reflectance is used instead of a mirror.

This is a method that is applied to solid samples, including fine particles, as well as liquids and suspended samples. The thickness of a sample must be adjusted when applying this method to a solid sample. Ordinarily adjustment is made by setting absorbance to 0.1 – 2 (transmittance of 79 – 1%), which provides the best linearity and SN ratio of a detector. A cell with appropriate layer length, according to the grain size of the fine particle, must be selected when applying the method to a fine particle sample.

3. Factors that affect spectra

Following items must be considered as factors that can affect spectra when applying near-infrared spectrometry, particularly when conducting quantitative analysis.

(i) Measurement conditions: A significant change (wavelength shift, for example) can occur when the temperature varies by a several degrees (°C). Care must be taken, particularly when a sample contains water. Also, water or residual solvent contents of a sample, as well as water (humidity) in the environment where in measurements are taken, can significantly affect absorption bands of the near-infrared range.

The thickness of a sample is a factor for spectral changes and therefore needs to be controlled at a certain thickness. Furthermore, since the condition of sample fill can potentially affect spectra when taking measurements of samples that are solids or fine particles, care must be taken with filling samples in a cell, to ensure that a certain amount is filled through a specific procedure.

Samples can potentially undergo chemical, physical or optical property changes, due to passing of time or storage after sampling. In creating calibration curves, therefore, it is necessary to pay attention that the samples for calibration curves must be prepared with adequate considerations for reducing the time to be measured, such as the measurement is carried out offline in a laboratory or online in manufacturing process (or inline).

(ii) Characteristics of samples: When a sample is physically, chemically or optically uneven, relatively large beam size must be used, multiple samples must be used, measurements must be taken at multiple points on the same samples, or a sample must be pulverized to ensure averaging of the sample. Grain size, fill condition, as well as roughness of surfaces can also affect the spectra of fine particle samples. Since variations in crystal structures (crystal polymorphism) can also affect spectra, in cases where multiple crystal forms exist, care must be taken to ensure that even standard samples for the calibration curve method have diversified distributions similar to that of samples to be analyzed.

4. Control of equipment performance

4.1. Accuracy of wavelengths (or wave numbers)

The accuracy of wavelengths (or wave numbers) of equipment is derived from the deviation of suitable substances for which peak absorption wavelengths (or wave numbers) have been defined, such as polystyrene, mixture of rare earth oxides (dysprosium, holmium and erbium; 1:1:1) or steam, from the figures indicated on the equipment. Tolerance figures in the vicinity of 3 peaks are ordinarily set in the following manner, though appropriate tolerance figures can be set depending on the intended purpose:

1200 ± 1 nm (8300 ± 8 cm^{-1})
1600 ± 1 nm (6250 ± 6 cm^{-1})
2000 ± 1.5 nm (5000 ± 4 cm^{-1})

Since the location of absorption peaks vary, depending on the substance used as reference, absorption peaks of wavelengths (or wave numbers) that are closest to the above 3 peaks are selected for suitability evaluation. A mixture of rare earth oxides, for instance, would indicate characteristic absorption peaks at 1261 nm (7930 cm^{-1}), 1681 nm (5949 cm^{-1}) and 1971 nm (5074 cm^{-1}).

The absorption peak of steam at 1368.6 nm (7306.7 cm^{-1}) can be used with a Fourier transformation type spectrophotometer, as its wave number resolution ability is high.

Other substances can also be used as the reference, so long as their adequacy for the purpose can be verified.

4.2. Spectroscopic linearity

Appropriate standard plates, such as plate-shaped polymer impregnated with varying concentrations of carbon (Carbon-doped polymer standards), can be used to evaluate spectroscopic linearity. In order to verify linearity, however, standard plates with no less than 4 levels of concentration within the reflectance of 10 – 90% must be used. When measurements are expected to be taken with absorbance of not less than 1.0, it is necessary to add standard plates with the reflectance of either 2% or 5% or both.

In order to plot absorbance of such standard plates at locations in the vicinity of wavelengths 1200 nm (8300 cm^{-1}), 1600 nm (6250 cm^{-1}) and 2000 nm (5000 cm^{-1}) against absorbance at each wavelength (or wave number) assigned to each standard plate, ensure that the gradient of linearity and ordinate intercept obtained are ordinarily within the ranges of 1.00 ± 0.05 and 0.00 ± 0.05, respectively. Depending on the intended purpose, appropriate tolerance figures can be set.

5. Application of qualitative or quantitative analysis

Ordinarily, chemometrics methods are used for analyzing a near-infrared absorption spectrum. Conventional spectrometric methods, such as a calibration curve method, may be used as a method whenever applicable. Chemometrics ordinarily involve the quantification of chemical data, as well as numerical and statistical procedures for computerization of information. Various types of multivariate analysis are used as chemometrics for near infrared spectrometry, and are selected according to the intended purpose. Characteristics of near-infrared absorption spectrum must be emphasized and effects of complexities of spectra, as well as overlay of absorption bands must be reduced by performing mathematical preprocesses, such as primary or secondary spectral differentiation processes or normalizations, which becomes one of vital procedures in establishing analysis methods that use methodologies of chemometrics.

In near-infrared spectroscopy, sustaining and managing performance of an analysis method, once established, are critical. Continuous and systematic maintenance and inspection work are required. Furthermore, it is necessary to pay attention to whether or not appropriate evaluation procedures are available to deal with change controls or implementation of re-validation on changes made in manufacturing processes or raw materials, as well as changes arising from replacement of major components in equipment.

5.1. Qualitative analysis

Qualitative analysis is performed for each substance to be analyzed after preparing a reference library that includes inter-lot variations within the tolerance range and establishing an analysis method using chemometrics methodology. The identity of substances can be confirmed by comparison with a standard spectrum or by methods using validated chemometrics software. Also, substances can be identified by their absorbance bands.

Furthermore, multivariate analysis includes direct analysis methods that consider wavelengths (or wave numbers) and absorption as variables, such as wavelength correlation method, residual sum of squares, range sum of squares, along with factor analysis method, cluster analysis method, discriminant analysis method and SIMCA (Soft independent modeling of class analogy) that are applied after processing such as principal component analysis.

It is also possible to consider the overall near-infrared absorption spectrum as a single pattern and to identify parameters obtained by applying multivariate analysis methods or characteristic wavelength (or wave number)

peaks of components to be analyzed as indices for monitoring, for the purpose of manufacturing process control for drug substances or preparations.

5.2. Quantitative analysis

Quantitative analysis ordinarily uses spectra of sample groups and analysis values obtained through the existing and established analysis methods, to obtain quantitative models with methodologies of chemometrics. These are used to calculate concentrations of individual ingredients and material values of samples being measured, using conversion formulas. Chemometrics methodologies for obtaining quantitative models include multiple regression analysis method and PLS (Partial least squares) regression analysis method.

In cases where the composition of a sample is simple, concentrations of ingredients in the sample to be analyzed can be calculated by plotting a calibration curve using the absorbance of a specific wavelength (or wave number) or the correlating relationship between the parameters and concentration, using samples for preparation of calibration curves with known concentrations (calibration curve method).

Add the following:

2.28 Circular Dichroism Spectroscopy

The circular dichroism spectroscopy is a method used to analyze and determine the structure of optically active substances, discriminate active substances from enantiomers, diastereomers, etc. by using the phenomenon (circular dichroism) in which the degrees of absorption of left and right circularly polarized lights differ in the absorption wavelength region of active substances.

In this method circular dichroism is measured as the difference of absorbance of left and right circularly polarized lights as follows.

$$\Delta A = A_L - A_R$$

ΔA: Difference of absorbance of left and right circularly polarized lights
A_L: Absorbance of left circularly polarized light
A_R: Absorbance of right circularly polarized light

Also, the difference of molar absorption coefficients for left and right circularly polarized lights can be expressed as the molar circular dichroism as follows.

$$\Delta\varepsilon = \varepsilon_L - \varepsilon_R = \frac{\Delta A}{c \times l}$$

$\Delta\varepsilon$: Molar circular dichroism $[(\mathrm{mol/L})^{-1}\cdot\mathrm{cm}^{-1}]$
ε_L: Molar absorption coefficient for left circularly polarized light $[(\mathrm{mol/L})^{-1}\cdot\mathrm{cm}^{-1}]$
ε_R: Molar absorption coefficient for right circularly polarized light $[(\mathrm{mol/L})^{-1}\cdot\mathrm{cm}^{-1}]$
c: Concentration of an optically active substance in solution (mol/L)
l: Path length (cm)

The following unit can also be used as the unit indicating circular dichroism.

Dissymmetry factor (g factor):

$$g = \frac{\Delta\varepsilon}{\varepsilon}$$

ε: Molar absorption coefficient

Molar ellipticity:

In some apparatuses, circular dichroism is expressed in units of ellipticity (°). In such a case, the molar ellipticity $[\theta]$ is calculated using the following equation.

$$[\theta] = \frac{\theta}{10 \times c \times l}$$

$[\theta]$: Molar ellipticity $(^\circ\cdot\mathrm{cm}^2/\mathrm{dmol})$
θ: Value (m°) of ellipticity calculated by apparatus
c: Concentration of an optically active substance in solution (mol/L)
l: Path length (cm)

Molar ellipticity is related with molar circular dichroism by the following equation.

$$[\theta] = 2.303\Delta\varepsilon\frac{4500}{\pi} \approx 3300\Delta\varepsilon$$

Molar circular dichroism and molar ellipticity are often used for analysis of peptides, proteins and nucleic acids. In this case, mean residue weight, which is the molecular mass divided by the number of monomeric residues, is used in the calculation of molar concentration (c).

$$\text{Mean residue weight} = \frac{\text{molecular mass}}{\text{number of amino acid residues or nucleotide residues}}$$

Mean residue weight is 100 – 120 (generally 115) for peptides and proteins, and is about 330 as sodium salt for nucleic acids.

1. Apparatus

A circular dichroism spectrophotometer is used. A xenon lamp is used as the light source. Light from the light source is polarized at the time when being split by a double monochromator equipped with a crystal prism, resulting in monochromatic linearly polarized light. The slit at the exit of the monochromator eliminates extraordinary light. The monochromatic linearly polarized light is passed through a photoelastic modulator to be alternately modulated into left and right circularly polarized lights at a constant frequency and is irradiated to a sample.

After the light that has passed through a sample to be tested reaches a photomultiplier tube, the light is divided into two electrical signals and amplified. One is the direct current signal, V_{DC}, which reflects the light absorption of the sample. The other is the alternating current signal, V_{AC}, which occurs when the sample has circular dichroism and has the same frequency as the modulation frequency of the

photoelastic modulator. The phase of the direct current signal indicates the sign of the circular dichroism (+ or −), and the magnitude of the amplitude indicates the intensity of the circular dichroism. Here, V_{AC}/V_{DC} is proportional to the difference of the absorbances for left and right circular polarized lights, ΔA. Generally, the wavelength range measured by a circular dichroism spectrophotometer is about 170 to 800 nm, but some apparatuses can measure at a wider wavelength range.

2. Methods

Set temperature, wavelength, path length and sample concentration for measurement. Dissolve a sample in an appropriate solvent, place it in a cell, and measure. In the sample preparation, confirm the influence of impurities on the spectrum, the structural change of the sample depending on the concentration, the absorption of the solvent itself, and the influence of the solvent on the sample structure. Attention should be taken for the optical path length of a sample cell, especially when the optical path length is short. Furthermore, it should be noted for the absorption of light by a sample because it may reduce a signal reaching a detector.

2.1. Identification test

Specify molar circular dichroism or molar ellipticity along with the wavelength at which it is maximum. The identity of a substance can be confirmed when the molar circular dichroism or molar ellipticity at the specified maximum wavelength of the substance to be confirmed meets this specification. Or, when the spectrum of a sample is compared with the reference spectrum of the substance to be confirmed or the spectrum of the reference standard, and both spectra give the same intensity of molar circular dichroism or molar ellipticity at the same wavelength, their identity can be confirmed mutually.

2.2. Analysis of secondary structure

For peptides and proteins, specific spectra appear in the far ultra-violet region. The secondary structure of peptides and proteins can be estimated by measuring the spectrum below about 250 nm. Furthermore, it is possible to estimate the three-dimensional structure from the near ultra-violet spectrum. However, it should be noted that circular dichroism measurement observes the average property of a whole molecule. For a α-helix structure, negative maxima appear generally at 208 nm and 222 nm and a positive maximum between 191 nm and 193 nm, for a β-sheet structure, a negative maximum appears between 216 nm and 218 nm and a positive maximum between 195 nm and 200 nm, and for an irregular structure, a negative maximum appears between 195 nm and 200 nm. Methods for analyzing the proportion of secondary structures from a circular dichroism spectrum include a method using a calculation formula and a method using a database. It can also be calculated by multivariate analysis. Whenever any method is used, the method used for the calculation is specified in the test method.

3. Verification of the performance of apparatus

A wavelength-calibrated apparatus is used, and the performance of the apparatus is verified using a sample with quality suitable for the measurement of circular dichroism and with known $\Delta\varepsilon$.

3.1. Accuracy of circular dichroism

Calibrate the accuracy of circular dichroism with a substance with known $\Delta\varepsilon$, such as isoandrosterone, ammonium *d*-camphorsulfonate, etc. (substances recommended by the apparatus manufacturer may be used). When using isoandrosterone, weigh exactly 10.0 mg of isoandrosterone, and dissolve in ethanol (99.5) to make exactly 10 mL. When the circular dichroism spectrum of the prepared solution is measured in the range of 280 nm to 360 nm using a cell with a path length of 10 mm, $\Delta\varepsilon$ at 304 nm is +3.3.

3.2. Linearity of modulation

Calibrate the linearity of modulation with a substance with known $\Delta\varepsilon$, such as ammonium *d*-camphorsulfonate (substances recommended by the apparatus manufacturer may be used). When using ammonium *d*-camphorsulfonate, weigh exactly 6.0 mg of ammonium *d*-camphorsulfonate and dissolve in water to make exactly 10 mL. When the circular dichroism spectrum of the prepared solution is measured in the range of 185 nm to 340 nm using a cell with a path length of 1 mm, $\Delta\varepsilon$ at 290.5 nm is +2.2 to +2.5 and $\Delta\varepsilon$ at 192.5 nm is −4.3 to −5.

Change the following as follows:

2.58 X-Ray Powder Diffraction Method

This test is harmonized with the European Pharmacopoeia and the U.S. Pharmacopeia.

The parts of the text that are not harmonized among the targeted texts for the harmonization are marked with symbols (◆ ◆), and the texts that are uniquely specified by the JP other than the targeted texts for the harmonization are marked with symbols (◇ ◇).

Information on the harmonization with the European Pharmacopoeia and the U.S. Pharmacopeia is available on the website of the Pharmaceuticals and Medical Devices Agency.

◇X-Ray Powder Diffraction Method is a method for measuring characteristic X-ray diffraction angles and intensities from randomly oriented powder crystallites irradiated by a monochromated X-ray beam.◇

Every crystalline phase of a given substance produces a characteristic X-ray diffraction pattern. Diffraction patterns can be obtained from a randomly oriented crystalline powder composed of crystallites (crystalline region within a particle) or crystal fragments of finite size. Essentially 3 types of information can be derived from a powder diffraction pattern: angular position of diffraction lines (depending on geometry and size of the unit cell); intensities of diffraction lines (depending mainly on atom type and arrangement, and preferred orientation within the sample); and diffraction line profiles (depending on instrumental resolution, crystallite size, strain and specimen thickness).

Experiments giving angular positions and intensities of lines can be used for applications such as qualitative phase analysis (for example, identification of crystalline phases) and quantitative phase analysis of crystalline materials. An estimate of the amorphous and crystalline fractions[1] can also be made. The X-ray powder diffraction (XRPD) method provides an advantage over other means of analysis in that it is usually non-destructive in nature (specimen preparation is usually limited to grinding to ensure a randomly oriented sample). XRPD investigations can also be carried out under *in situ* conditions on specimens exposed to non-ambient conditions, such as low or high temperature and humidity.

1. Principle

X-ray diffraction results from the interaction between X-rays and electron clouds of atoms. Depending on the atomic arrangement, interferences arise from the elastically scattered X-rays. These interferences are constructive when the path difference between 2 diffracted X-ray waves differs by an integral number of wavelengths. This selective condition is described by the Bragg equation, also called Bragg's law (see Fig. 2.58-1)

$$2d_{hkl} \sin \theta_{hkl} = n\lambda$$

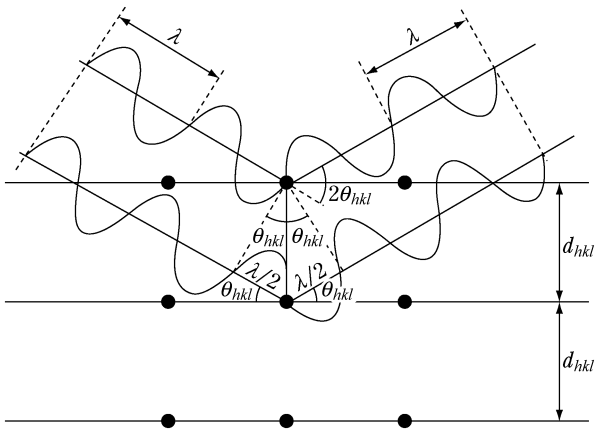


Fig. 2.58-1 Diffraction of X-rays by a crystal according to Bragg's law

The wavelength λ of the X-rays is of the same order of magnitude as the distance between successive crystal lattice planes, or d_{hkl} (also called '*d*-spacings'). θ_{hkl} is the angle between the incident ray and the family of lattice planes, and $\sin\theta_{hkl}$ is inversely proportional to the distance between successive crystal planes or *d*-spacings.

The direction and spacing of the planes with reference to the unit cell axes are defined by the Miller indices $\{hkl\}$. These indices are the reciprocals, reduced to the next-lower integer, of the intercepts that a plane makes with the unit cell axes. The unit cell dimensions are given by the spacings, *a*, *b* and *c* and the angles between them, α, β and γ. The interplanar spacing for a specified set of parallel *hkl* planes is denoted by d_{hkl}. Each such family of planes may show higher orders of diffraction where the *d* values for the related families of planes, *nh*, *nk*, *nl* are diminished by the factor $1/n$ (*n* being an integer: 2,3,4, etc.). Every set of planes throughout a crystal has a corresponding Bragg diffraction angle, θ_{hkl}, associated with it (for a specific wavelength λ).

For polycrystalline powder specimen at any angle θ_{hkl} there are always crystallites in an orientation allowing diffraction according to Bragg's law[2]. For a given X-ray wavelength, the positions of the diffraction peaks (also referred to as 'lines', 'reflections' or 'Bragg reflections') are characteristic of the crystal lattice (*d*-spacings), their theoretical intensities depend on the crystallographic unit cell content (nature and positions of atoms), and the line profiles on the perfection and extent of the crystal lattice. Under these conditions the diffraction peak has a finite intensity arising from atomic arrangement, type of atoms, thermal motion and structural imperfections, as well as from instrument characteristics. The intensity is also dependent upon many factors such as structure factor, temperature factor, polarization factor, multiplicity, Lorentz factor and microabsorption factors. The main characteristics of diffraction line profiles are 2θ position, peak height, peak area and shape (characterized by, for example, peak width or asymmetry, analytical function, empirical representation). An example of the type of powder patterns obtained for 5 different solid phases of a substance is shown in Fig. 2.58-2.

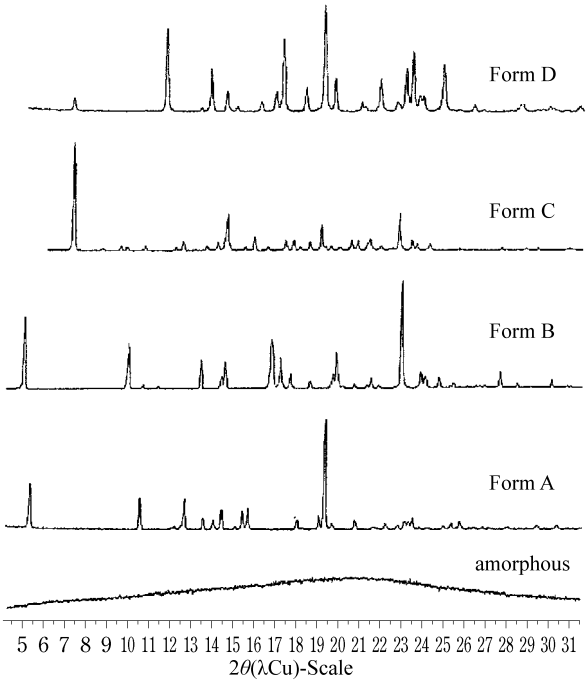


Fig. 2.58-2 X-ray powder diffraction patterns collected for 5 different solid phases of a substance (the intensities of crystalline forms A-D are normalized)

In addition to the diffraction peaks, an X-ray diffraction experiment also generates a more-or-less uniform background, upon which the peaks are superimposed. Besides

specimen preparation, other factors contribute to the background, for instance the sample holder, diffuse scattering from air, the sample and/or the equipment, other instrumental parameters such as detector noise, general radiation from the X-ray tube, etc. The peak to background ratio can be increased by minimizing background and by choosing prolonged exposure times.

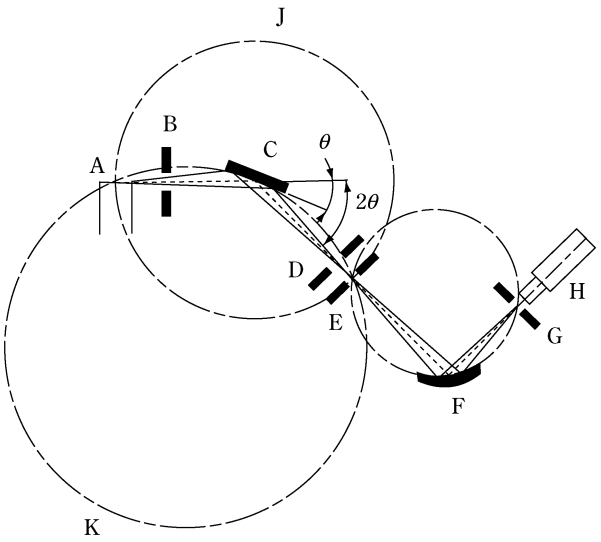
2. Instrument

2.1. Instrument set-up

X-ray diffraction experiments are usually performed using powder diffractometers or powder cameras. A powder diffractometer generally comprises 5 main parts: an X-ray source; incident beam optics, which may perform monochromatization, filtering, collimation and/or focusing of the beam; a goniometer; the diffracted beam optics, which may perform monochromatization, filtering, collimation and/or focusing of the beam; and a detector. Data-collection and data-processing systems are also required and are generally included with current diffraction measurement equipment.

Depending on the type of analysis to be performed (phase identification, quantitative analysis, lattice parameters determination, etc.), different XRPD instrument configurations and performance levels are required. The simplest instruments used to measure XRPD patterns are powder cameras. The replacement of photographic film as the detection method by photon detectors has led to the design of diffractometers in which the geometric arrangement of the optics is not truly focusing but parafocusing, such as in the Bragg-Brentano geometry. The Bragg-Brentano parafocusing configuration is currently the most widely used and is therefore briefly described here.

A given instrument may provide a horizontal or vertical $\theta/2\theta$ geometry or a vertical θ/θ geometry. For both geometries, the incident X-ray beam forms an angle θ with the specimen surface plane and the diffracted X-ray beam forms an angle 2θ with the direction of the incident X-ray beam (an angle θ with the specimen surface plane). One example of a basic geometric arrangement is represented in Fig. 2.58-3. The divergent beam of radiation from the X-ray tube (the so-called 'primary beam') passes through the Soller slit and a divergence slit assembly and illuminates the flat surface of the specimen. All the rays diffracted by suitably oriented crystallites in the specimen at an angle 2θ converge to a line at the receiving slit. A second set of Soller slit and a scatter slit may be placed either behind or before the receiving slit; the receiving slit is normally only used when a 0D detector is present. The axes of the line focus and of the receiving slit are at equal distances from the axis of the goniometer. The X-rays are counted by a detector, usually a scintillation counter, or a sealed-gas proportional counter. However, nowadays a position-sensitive solid-state detector or hybrid photon counting detectors are more common. The receiving slit assembly and the detector are coupled together and move tangentially to the focusing circle. For $\theta/2\theta$ scans the goniometer rotates the specimen about the same axis as that of the detector, but at half the rotational speed, in a $\theta/2\theta$ motion. The surface of the specimen thus remains tangential to the focusing circle. The Soller slit limits the axial divergence of the beam and hence partially controls the shape of the diffracted line profile.



A. X-ray tube
B. Divergence slit
C. Sample
D. Anti-diffusion slit
E. Receiving slit
F. Monochromator
G. Detector receiving slit
H. Detector
J. Diffractometer circle
K. Focusing circle

Fig. 2.58-3 Geometric arrangement of the Bragg-Brentano parafocusing geometry

A diffractometer may also be used in transmission mode. The advantage with this technology is to lessen the effects due to preferred orientation. A capillary of about 0.5 – 2 mm thickness can also be used for small sample amounts.

2.2. X-ray radiation

In the laboratory, X-rays are obtained by bombarding a metal anode with electrons emitted by the thermionic effect and accelerated in a strong electric field (using a high-voltage generator). Most of the kinetic energy of the electrons is converted to heat, which limits the power of the tubes and requires efficient anode cooling. A 20- to 30-fold increase in brilliance can be obtained using rotating anodes and by using X-ray optics. Alternatively, X-ray photons may be produced in a large-scale facility (synchrotron).

The spectrum emitted by an X-ray tube operating at sufficient voltage consists of a continuous background of polychromatic radiation (Bremsstrahlung or white radiation) and additional characteristic radiation that depends on the type of anode. Normally only this characteristic radiation is used in X-ray diffraction experiments. The principal radiation sources utilized for X-ray diffraction are vacuum tubes utilizing copper, molybdenum, iron, cobalt, silver or chromium as anodes; copper and molybdenum X-rays are employed most commonly for organic substances. The choice of radiation to be used depends on the absorption

characteristics of the specimen and possible fluorescence by atoms present in the specimen. The wavelengths used in powder diffraction generally correspond to the K_α radiation from the anode. Consequently, it is advantageous to make the X-ray beam 'monochromatic' by eliminating all the other components of the emission spectrum. This can be partly obtained using K_β filters, i.e. metal filters selected as having an absorption edge between the K_α and K_β wavelengths emitted by the tube.

Such a filter is usually inserted between the X-ray tube and the specimen. Another, more-and-more-commonly used way to obtain a monochromatic X-ray beam is via a large monochromator crystal (usually referred to as a 'monochromator'). This crystal is placed before or behind the specimen and diffracts the different characteristic peaks of the X-ray beam (i.e. K_α and K_β) at different angles, so that only one of them may be selected to enter into the detector. It is even possible to separate $K_{\alpha1}$ and $K_{\alpha2}$ radiations by using a specialized monochromator. Unfortunately, the gain in getting a monochromatic beam by using a filter or a monochromator is counteracted by a loss in intensity. Another way of separating K_α and K_β wavelengths is by using curved X-rays mirrors that can simultaneously monochromate and focus or parallelize the X-ray beam.

2.3. Radiation protection

Exposure of any part of the human body to X-rays can be injurious to health. It is therefore essential that whenever X-ray equipment is used, adequate precautions are taken to protect the operator and any other person in the vicinity. Recommended practice for radiation protection as well as limits for the levels of X-radiation exposure are those established by national legislation in each country. If there are no official regulations or recommendations in a country, the latest recommendations of the International Commission on Radiological Protection should be applied.

3. Specimen preparation and mounting

The preparation of the powdered material and mounting of the specimen in a suitable holder are critical steps in many analytical methods, and are particularly so for XRPD analysis, since they can greatly affect the quality of the data to be collected[3]. The main sources of error due to specimen preparation and mounting are briefly discussed here for instruments in Bragg-Brentano parafocusing geometry.

3.1. Specimen preparation

In general, the morphology of many crystalline particles tends to give a specimen that exhibits some degree of preferred orientation in the specimen holder. This is particularly evident for needle-like or plate-like crystals when size reduction yields finer needles or platelets. Preferred orientation in the specimen influences the intensities of various reflections, so that some are more intense and others are less intense, compared to what would be expected from a completely random specimen. Several techniques can be employed to improve randomness in the orientation of crystallites (and therefore to minimize preferred orientation), but further reduction of particle size is often the best and simplest approach. The optimum number of crystallites depends on the diffractometer geometry, the required resolution and the specimen attenuation of the X-ray beam. In some cases, particle sizes as large as 50 μm will provide satisfactory results in phase identification. However, excessive milling (particle sizes less than approximately 0.5 μm) may cause line broadening and significant changes to the sample itself such as:

(i) specimen contamination by particles abraded from the milling instruments (mortar, pestle, balls, etc.);
(ii) reduced degree of crystallinity;
(iii) solid-state transition to another polymorph;
(iv) chemical decomposition;
(v) introduction of internal stress;
(vi) solid-state reactions.

Therefore, it is advisable to compare the diffraction pattern of the non-ground specimen with that corresponding to a specimen of smaller particle size (e.g. a milled specimen). If the XRPD pattern obtained is of adequate quality considering its intended use, then grinding may not be required. It should be noted that if a sample contains more than one phase and if sieving is used to isolate particles to a specific size, the initial composition may be altered.

4. Control of the instrument performance

Goniometers and the corresponding incident and diffracted X-ray beam optics have many mechanical parts that need adjustment. The degree of alignment or misalignment directly influences the quality of the results of an XRPD investigation. Therefore, the different components of the diffractometer must be carefully adjusted (optical and mechanical systems, etc.) to minimize adequately systematic errors, while optimizing the intensities received by the detector. The search for maximum intensity and maximum resolution is always antagonistic when aligning a diffractometer. Hence, the best compromise must be sought whilst performing the alignment procedure. There are many different configurations and each supplier's equipment requires specific alignment procedures.

The overall diffractometer performance must be tested and monitored periodically using suitable certified reference materials, e.g. silicon powder or α-alumina (corundum). Depending on the type of analysis, other well-defined reference materials may also be employed, although the use of certified reference materials is preferred.

5. Qualitative phase analysis (Identification of phases)

The identification of the phase composition of an unknown sample by XRPD is usually based on the visual or computer-assisted comparison of a portion of its XRPD pattern to the experimental or calculated pattern of a reference material. Ideally, these reference patterns are collected on well-characterized single-phase specimens. This approach makes it possible in most cases to identify a crystalline substance by its 2θ diffraction angles or d-spacings and by its relative intensities. The computer-aided comparison of the diffraction pattern of the unknown sample to the reference data can be based either on a more-or-less extended 2θ-range of the whole diffraction pattern or on a set of reduced data derived from the pattern. For example, the list

of *d*-spacings and normalized intensities I_{norm}, a so-called (d, I_{norm})-list extracted from the pattern, is the crystallographic fingerprint of the material, and can be compared to (d, I_{norm})-lists of single-phase samples compiled in databases.

For most organic crystals, when using Cu K_α radiation, it is appropriate to record the diffraction pattern in a 2θ-range from as near 0° as possible to at least 30°. The agreement in the 2θ-diffraction angles between specimen and reference is expected to be within 0.2° for the same crystal form, while relative intensities between specimen and reference may vary considerably due to preferred orientation effects. By their very nature, variable hydrates and solvates are recognized to have varying unit cell dimensions and as such shifting occurs in peak positions of the measured XRPD patterns for these materials. In these unique materials, variance in 2θ-positions of greater than 0.2° is not unexpected. As such, peak position variances such as 0.2° are not applicable to these materials. For other types of samples (e.g. inorganic salts), it may be necessary to extend the 2θ-region scanned to well beyond 30°. It is generally sufficient to scan past the 10 strongest reflections identified in single phase XRPD database files.

It is sometimes difficult or even impossible to identify phases in the following cases:

(i) non-crystallized or amorphous substances;

(ii) the components to be identified are present in low mass fractions of the analyte amounts (generally less than 10 per cent *m/m*);

(iii) pronounced preferred orientation effects;

(iv) the phase has not been filed in the database used;

(v) formation of solid solutions;

(vi) presence of disordered structures that alter the unit cell;

(vii) the specimen comprises too many phases;

(viii) presence of lattice deformations;

(ix) structural similarity of different phases.

6. Quantitative phase analysis

If the sample under investigation is a mixture of 2 or more known phases, of which not more than 1 is amorphous, the percentage (by volume or by mass) of each crystalline phase and of the amorphous phase can, in many cases, be determined. Quantitative phase analysis can be based on the integrated intensities, on the peak heights of several individual diffraction lines[4], or on the full pattern. These integrated intensities, peak heights or full-pattern data points are compared to the corresponding values of reference materials. These reference materials shall be single-phase or a mixture of known phases. The difficulties encountered during quantitative analysis are due to specimen preparation (the accuracy and precision of the results require in particular homogeneity of all phases and a suitable particle size distribution in each phase) and to matrix effects. Amounts of crystalline phases as small as 10 per cent may usually be determined in solid matrices, and in favorable cases amounts of crystalline phases less than 10 per cent may be determined.

6.1. Polymorphic samples

For a sample composed of 2 polymorphic phases a and b, the following expression may be used to quantify the fraction F_a of phase a:

$$F_a = \frac{1}{1 + K\,(I_b/I_a)}$$

The fraction is derived by measuring the intensity ratio between the 2 phases, knowing the value of the constant K. K is the ratio of the absolute intensities of the 2 pure polymorphic phases I_{oa}/I_{ob}. Its value can be determined by measuring standard samples.

6.2. Methods using a standard

The most commonly used methods for quantitative analysis are:

– the 'external standard method';

– the 'internal standard method';

– the 'spiking method' (often also called the 'standard addition method').

The 'external standard method' is the most general method and consists of comparing the X-ray diffraction pattern of the mixture, or the respective line intensities, with those measured in a reference mixture or with the theoretical intensities of a structural model, if it is fully known.

To limit errors due to matrix effects, an internal reference material with crystallite size and X-ray absorption coefficient comparable to those of the components of the sample, and with a diffraction pattern that does not overlap at all that of the sample to be analyzed, can be used. A known quantity of this reference material is added to the sample to be analyzed and to each of the reference mixtures. Under these conditions, a linear relationship between line intensity and concentration exists. This application, called the 'internal standard method', requires a precise measurement of diffraction intensities.

In the 'spiking method' (or 'standard addition method'), some of the pure phase a is added to the mixture containing the unknown concentration of the phase a. Multiple additions are made to prepare an intensity-versus-concentration plot in which the negative x intercept is the concentration of the phase a in the original sample.

7. Estimate of the amorphous and crystalline fractions

In a mixture of crystalline and amorphous phases, the crystalline and amorphous fractions can be estimated in several ways. The choice of the method used depends on the nature of the sample:

(i) if the sample consists of crystalline fractions and an amorphous fraction of different chemical compositions, the amounts of each of the individual crystalline phases may be estimated using appropriate standard substances as described above; the amorphous fraction is then deduced indirectly by subtraction;

(ii) if the sample consists of one amorphous and one crystalline fraction, either as a 1-phase or a 2-phase mixture, with the same elemental composition, the amount of the crystalline phase ('the degree of crystallinity') can be estimated by measuring 3 areas of the diffractogram:

A: Sum of the area of all the peaks arising from diffraction of the crystalline fraction of the sample:
B: Area under the diffractogram generated by the sample (excluding area A);
C: Area of the background noise (due to air scattering, fluorescence, equipment, etc.)

When these areas have been measured, the degree of crystallinity can be roughly estimated using the following formula:

$$\%\text{crystallinity} = 100A/(A + B - C)$$

It is noteworthy that this method does not yield absolute degree-of-crystallinity values and hence is generally used for comparative purposes only. More sophisticated methods are also available, such as the Ruland method.

8. Single crystal structure

In general, the determination of crystal structures is performed from X-ray diffraction data obtained using single crystals. However, crystal structure analysis of organic crystals is a challenging task, since the lattice parameters are comparatively large, the symmetry is low and the scattering properties are normally very low. For any given crystalline form of a substance, knowledge of the crystal structure allows the calculation of the corresponding XRPD pattern, thereby providing a 'preferred-orientation-free' reference XRPD pattern, which may be used for phase identification.

(1) There are many other applications of the X-ray powder diffraction technique that can be applied to crystalline pharmaceutical substances, such as determination of crystal structures, refinement of crystal structures, determination of crystallographic purity of crystalline phases, characterization of crystallographic texture, etc. These applications are not described in this chapter.

(2) An 'ideal' powder for diffraction experiments consists of a large number of small, randomly oriented spherical particles (coherently diffracting crystalline domains). If this number is sufficiently large, there are always enough crystallites in any diffracting orientation to give reproducible diffraction patterns.

(3) Similarly, changes in the specimen can occur during data collection in the case of a nonequilibrium specimen (temperature, humidity).

(4) If the crystal structures of all components are known, the Rietveld method can be used to quantify them with good accuracy. If the crystal structures of the components are not known, the Pawley or least squares methods can be used.

3.04 Particle Size Determination

Change the 2.1. Procedure and below as follows:

2.1. Procedure

2.1.1 Test Sieves

Unless otherwise specified in the monograph, use those sieves listed in the Table 3.04-1 as recommended in the particular region.

Sieves are selected to cover the entire range of particle sizes present in the test specimen. A nest of sieves having a $\sqrt{2}$ progression of the area of the sieve openings is recommended. The nest of sieves is assembled with the coarsest screen at the top and the finest at the bottom. Use micrometers or millimeters in denoting test sieve openings. [Note—Sieve numbers are provided in the table for conversion purposes only.] Test sieves are made from stainless steel or, less preferably, from brass or other suitable nonreactive wire.

2.1.1.1. Calibration of test sieves

Calibration and recalibration of test sieves is in accordance with the most current edition of ISO 3310-1[2]. Sieves should be carefully examined for gross distortions and fractures, especially at their screen frame joints, before use. Sieves may be calibrated optically to estimate the average opening size, and opening variability, of the sieve mesh. Alternatively, for the evaluation of the effective opening of test sieves in the size range of 212 to 850 μm, Standard Glass Spheres are available. Unless otherwise specified in the individual monograph, perform the sieve analysis at controlled room temperature and at ambient relative humidity.

2.1.1.2. Cleaning Test Sieves

Ideally, test sieves should be cleaned using only an air jet or a liquid stream. If some apertures remain blocked by test particles, careful gentle brushing may be used as a last resort.

2.1.2. Test Specimen

If the test specimen weight is not given in the monograph for a particular material, use a test specimen having a weight between 25 and 100 g, depending on the bulk density of the material, and test sieves having a diameter of 200 mm or 203 mm (8 inch). For sieves of 75 mm or 76 mm (3-inch diameter) the amount of material that can be accommodated is approximately 1/7th of which can be accommodated on a 200 mm or 203 mm sieve. Determine the most appropriate weight for a given material by test sieving accurately weighed specimens of different weights, such as 25, 50, and 100 g, for the same time period on a mechanical shaker. [Note—If the test results are similar for the 25-g and 50-g specimens, but the 100-g specimen shows a lower percentage through the finest sieve, the 100-g specimen size is too large.] Where only a specimen of 10 to 25 g is available, smaller diameter test sieves conforming to the same mesh specifications may be substituted, but the endpoint must be re-determined. The use of test samples having a smaller mass (e.g. down to 5 g) may be needed. For materials with

Table 3.04-1 Sizes of standard sieve series in range of interest

ISO Nominal Aperture			US Sieve No.	Recommended USP Sieves (microns)	European Sieve No.	Japan Sieve No.
Principal sizes	Supplementary sizes					
R 20/3	R 20	R 40/3				
11.20 mm	11.20 mm 10.00 mm	11.20 mm			11200	
		9.50 mm				
8.00 mm	9.00 mm 8.00 mm 7.10 mm	8.00 mm				
		6.70 mm				
5.60 mm	6.30 mm 5.60 mm 5.00 mm	5.60 mm			5600	3.5
		4.75 mm				4
4.00 mm	4.50 mm 4.00 mm 3.55 mm	4.00 mm	5	4000	4000	4.7
		3.35 mm	6			5.5
2.80 mm	3.15 mm 2.80 mm 2.50 mm	2.80 mm	7	2800	2800	6.5
		2.36 mm	8			7.5
2.00 mm	2.24 mm 2.00 mm 1.80 mm	2.00 mm	10	2000	2000	8.6
		1.70 mm	12			10
1.40 mm	1.60 mm 1.40 mm 1.25 mm	1.40 mm	14	1400	1400	12
		1.18 mm	16			14
1.00 mm	1.12 mm 1.00 mm 900 μm	1.00 mm	18	1000	1000	16
		850 μm	20			18
710 μm	800 μm 710 μm 630 μm	710 μm	25	710	710	22
		600 μm	30			26
500 μm	560 μm 500 μm 450 μm	500 μm	35	500	500	30
		425 μm	40			36
355 μm	400 μm 355 μm 315 μm	355 μm	45	355	355	42
		300 μm	50			50
250 μm	280 μm 250 μm 224 μm	250 μm	60	250	250	60
		212 μm	70			70
180 μm	200 μm 180 μm 160 μm	180 μm	80	180	180	83
		150 μm	100			100
125 μm	140 μm 125 μm 112 μm	125 μm	120	125	125	119
		106 μm	140			140
90 μm	100 μm 90 μm 80 μm	90 μm	170	90	90	166
		75 μm	200			200
63 μm	71 μm 63 μm 56 μm	63 μm	230	63	63	235
		53 μm	270			282
45 μm	50 μm 45 μm 40 μm	45 μm	325	45	45	330
		38 μm			38	391

The JP Drugs are to be tested according to the provisions given in the pertinent monographs, General Notices, General Rules for Crude Drugs, General Rules for Preparations, and General Tests for their conformity to the Japanese Pharmacopoeia. (*See the General Notices 5.*)

low apparent particle density, or for materials mainly comprising particles with a highly iso-diametrical shape, specimen weights below 5 g for a 200 mm or 203 mm sieve may be necessary to avoid excessive blocking of the sieve. During validation of a particular sieve analysis method, it is expected that the problem of sieve blocking will have been addressed.

If the test material is prone to picking up or losing significant amounts of water with varying humidity, the test must be carried out in an appropriately controlled environment. Similarly, if the test material is known to develop an electrostatic charge, careful observation must be made to ensure that such charging is not influencing the analysis. An antistatic agent, such as colloidal silicon dioxide and/or aluminum oxide, may be added at a 0.5 percent (m/m) level to minimize this effect. If both of the above effects cannot be eliminated, an alternative particle-sizing technique must be selected.

2.1.3. Agitation Methods

Several different sieve and powder agitation devices are commercially available, all of which may be used to perform sieve analyses. However, the different methods of agitation may give different results for sieve analyses and endpoint determinations because of the different types and magnitude of the forces acting on the individual particles under test. Methods using mechanical agitation or electromagnetic agitation, and that can induce either a vertical oscillation or a horizontal circular motion, or tapping or a combination of both tapping and horizontal circular motion are available. Entrainment of the particles in an air stream may also be used. The results must indicate which agitation method was used and the agitation parameters used (if they can be varied), since changes in the agitation conditions will give different results for the sieve analysis and endpoint determinations, and may be sufficiently different to give a failing result under some circumstances.

2.1.4. Endpoint Determination

The test sieving analysis is complete when the weight on any of the test sieves does not change by more than 5% or 0.1 g (10% in the case of 75 mm or 76 mm sieves) of the previous weight on that sieve. If less than 5% of the total specimen weight is present on a given sieve, the endpoint for that sieve is increased to a weight change of not more than 20% of the previous weight on that sieve.

If more than 50% of the total specimen weight is found on any one sieve, unless this is indicated in the monograph, the test should be repeated, but with the addition to the sieve nest of a more coarse sieve intermediate between that carrying the excessive weight and the next coarsest sieve in the original nest, i.e., addition of the ISO series sieve omitted from the nest of sieves.

2.2. Sieving Methods

2.2.1. Mechanical Agitation (Dry Sieving Method)

Tare each test sieve to the nearest 0.1 g. Place an accurately weighed quantity of test specimen on the top (coarsest) sieve, and replace the lid. Agitate the nest of sieves for 5 minutes. Then carefully remove each from the nest without loss of material. Reweigh each sieve, and determine the weight of material on each sieve. Determine the weight of material in the collecting pan in a similar manner. Reassemble the nest of sieves, and agitate for 5 minutes. Remove and weigh each sieve as previously described. Repeat these steps until the endpoint criteria are met (*see Endpoint Determination under Test Sieves*). Upon completion of the analysis, reconcile the weights of material. Total losses must not exceed 5% of the weight of the original test specimen.

Repeat the analysis with a fresh specimen, but using a single sieving time equal to that of the combined times used above. Confirm that this sieving time conforms to the requirements for endpoint determination. When this endpoint has been validated for a specific material, then a single fixed time of sieving may be used for future analyses, providing the particle size distribution falls within normal variation.

If there is evidence that the particles retained on any sieve are aggregates rather than single particles, the use of mechanical dry sieving is unlikely to give good reproducibility, a different particle size analysis method should be used.

2.2.2. Air Entrainment Methods (Air Jet and Sonic Shifter Sieving)

Different types of commercial equipment that use a moving air current are available for sieving. A system that uses a single sieve at a time is referred to as *air jet* sieving. It uses the same general sieving methodology as that described under the *Dry Sieving Method*, but with a standardized air jet replacing the normal agitation mechanism. It requires sequential analyses on individual sieves starting with the finest sieve to obtain a particle size distribution. Air jet sieving often includes the use of finer test sieves than used in ordinary dry sieving. This technique is more suitable where only oversize or undersize fractions are needed.

In the *sonic sifting* method, a nest of sieves is used, and the test specimen is carried in a vertically oscillating column of air that lifts the specimen and then carries it back against the mesh openings at a given number of pulses per minute. It may be necessary to lower the sample amount to 5 g, when sonic sifting is employed.

The air jet sieving and sonic sieving methods may be useful for powders or granules when mechanical sieving techniques are incapable of giving a meaningful analysis.

These methods are highly dependent upon proper dispersion of the powder in the air current. This requirement may be hard to achieve if the method is used at the lower end of the sieving range (i.e., below 75 μm), when the particles tend to be more cohesive, and especially if there is any tendency for the material to develop an electrostatic charge. For the above reasons endpoint determination is particularly critical, and it is very important to confirm that the oversize material comprises single particles and is not composed of aggregates.

2.3. Interpretation

The raw data must include the weight of test specimen, the total sieving time, and the precise sieving methodology and the set values for any variable parameters, in addition to the weights retained on the individual sieves and in the pan. It may be convenient to convert the raw data into a cu-

mulative weight distribution, and if it is desired to express the distribution in terms of a cumulative weight undersize, the range of sieves used should include a sieve through which all the material passes. If there is evidence on any of the test sieves that the material remaining on it is composed of aggregates formed during the sieving process, the analysis is invalid.

[1)]Additional information on particle size measurement, sample size, and data analysis is available, for example, in ISO 9276.
[2)]International Organization for Standardization (ISO) Specification ISO 3310-1: Test sieves-Technical requirements and testing—Part 1: Test sieves of metal wire cloth.

9.01 Reference Standards

Add the following to Section (1):

Anastrozole RS

Budesonide RS

Temozolomide RS

Delete the following from section (2), and add them to section (1):

Amikacin Sulfate RS

Cefaclor RS

Cefalexin RS

Clindamycin Phosphate RS

Doxorubicin Hydrochloride RS

Delete the following:

Nartograstim RS

9.41 Reagents, Test Solutions

Add the following:

1,4-Diaminobutane $C_4H_{12}N_2$ White to slightly pale yellow powder or masses, or colorless to pale yellow clear liquid.

Lead tetraacetate $Pb(CH_3COO)_4$ White to pale brown powder. Melting point: about 176°C (with decomposition).

Lead tetraacetate-fluorescein sodium TS To 5 mL of a solution of lead tetraacetate in acetic acid (100) (3 in 100) and 2.5 mL of a solution of fluorescein sodium in ethanol (99.5) (1 in 100) add dichloromethane to make 100 mL. Prepare before use.

Nootkatone for thin-layer chromatography $C_{15}H_{22}O$ White to pale yellow, crystals or crystalline powder. Very soluble in methanol, in ethanol (99.5) and in hexane, and practically insoluble in water.

Identification—Determine the infrared absorption spectrum of nootkatone for thin-layer chromatography as directed in the potassium bromide disk method as directed under Infrared Spectrophotometry <*2.25*>: it exhibits absorption at the wave numbers of about 2950 cm^{-1}, 1670 cm^{-1}, and 898 cm^{-1}.

Purity Related substances—Dissolve 2 mg of nootkatone for thin-layer chromatography in 2 mL of hexane, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add hexane to make exactly 20 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-Layer Chromatography <*2.03*>. Perform the test with 10 μL each of the sample solution and standard solution as directed in the Identification under Bitter Cardamon: the spots other than the principal spot with an *R*f value of about 0.35 obtained from the sample solution are not more intense than the spot from the standard solution.

Phosphate buffer solution (pH 3.2) To 900 mL of a solution of sodium dihydrogen phosphate dihydrate (1 in 250) add 100 mL of diluted phosphoric acid (1 in 400), and adjust to pH 3.2 with phosphoric acid or sodium hydroxide TS.

Potassium phosphate trihydrate $K_3PO_4.3H_2O$ White, crystalline powder or powder. Freely soluble in water. The pH of a solution of potassium phosphate trihydrate (1 in 100) is between 11.5 and 12.5.

Identification—(1) A solution of potassium phosphate trihydrate (1 in 20) responds to Qualitative Tests <*1.09*> (3) for potassium salt.
(2) A solution of potassium phosphate trihydrate (1 in 20) responds to Qualitative Tests <*1.09*> (1) for phosphate.

Temozolomide $C_6H_6N_6O_2$ [Same as the namesake monograph]

Change the following as follows:

Amygdalin for assay $C_{20}H_{27}NO_{11}$ Amygdalin for thin-layer chromatography. It meets the requirement of the following amygdalin for assay 1 or amygdalin for assay 2 (Purity value by quantitative NMR). The former is used after drying in a desiccator (silica gel) for 24 hours, and the latter is used with correction for its amount based on the result obtained in the Assay 2.

1) Amygdalin for assay 1

Absorbance <*2.24*> $E^{1\%}_{1\,cm}$ (263 nm): 5.2 – 5.8 [20 mg calculated on the anhydrous basis, methanol, 20 mL; separately determine the water <*2.48*> (5 mg, coulometric titration)].

Purity Related substances—Dissolve 5 mg of amygdalin for assay 1 in 10 mL of the mobile phase, and use this solu-

tion as the sample solution. Pipet 1 mL of the sample solution, add the mobile phase to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine each peak area by the automatic integration method: the total area of the peaks other than amygdalin from the sample solution is not larger than the peak area of amygdalin from the standard solution.

Operating conditions

Detector, column, column temperature, mobile phase and flow rate: Proceed as directed in the operating conditions in the Assay (3) under Keishibukuryogan Extract.

Time span of measurement: About 3 times as long as the retention time of amygdalin.

System suitability

Test for required detectability: Pipet 1 mL of the standard solution, and add the mobile phase to make exactly 20 mL. Confirm that the peak area of amygdalin obtained with 10 μL of this solution is equivalent to 3.5 to 6.5% of that with 10 μL of the standard solution.

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of amygdalin are not less than 5000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of amygdalin is not more than 1.5%.

2) Amygdalin for assay 2 (Purity value by quantitative NMR)

Unity of peak—Dissolve 1 mg of amygdalin for assay 2 in 5 mL of diluted methanol (1 in 2), and use this solution as the sample solution. Perform the test with 10 μL of the sample solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and compare the absorption spectra of at least 3 points including the top of amygdalin peak and around the two middle peak heights of before and after the top: no difference in form is observed among their spectra.

Operating conditions

Column, column temperature, mobile phase and flow rate: Proceed as directed in the operating conditions in the Assay (3) under Keishibukuryogan Extract.

Detector: A photodiode array detector (wavelength: 210 nm, measuring range of spectrum: 200 – 400 nm).

System suitability

System performance: When the procedure is run with 10 μL of the sample solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of amygdalin are not less than 5000 and not more than 1.5, respectively.

Assay—Weigh accurately 5 mg of amygdalin for assay 2 and 1 mg of DSS-d_6 for nuclear magnetic resonance spectroscopy using an ultramicrobalance, dissolve in 1 mL of deuterated dimethylsulfoxide for nuclear magnetic resonance spectroscopy, and use this solution as the sample solution. Transfer the sample solution into an NMR tube 5 mm in outer diameter, and measure ^{1}H-NMR as directed under Nuclear Magnetic Resonance Spectroscopy <*2.21*> and Crude Drugs Test <*5.01*> according to the following conditions, using DSS-d_6 for nuclear magnetic resonance spectroscopy as the reference standard for qNMR. Calculate the resonance intensity A (equivalent to 1 hydrogen) of the signal around δ 6.03 ppm assuming the signal of the reference standard for qNMR as δ 0 ppm.

$$\text{Amount (\%) of amygdalin } (C_{20}H_{27}NO_{11}) = M_S \times I \times P/(M \times N) \times 2.0388$$

M: Amount (mg) of amygdalin for assay 2 taken

M_S: Amount (mg) of DSS-d_6 for nuclear magnetic resonance spectroscopy taken

I: Signal resonance intensity A based on the signal resonance intensity of DSS-d_6 for nuclear magnetic resonance spectroscopy as 9.000

N: Number of the hydrogen derived from A

P: Purity (%) of DSS-d_6 for nuclear magnetic resonance spectroscopy

Operating conditions

Apparatus: A nuclear magnetic resonance spectrometer having ^{1}H resonance frequency of not less than 400 MHz.

Target nucleus: ^{1}H.

Digital resolution: 0.25 Hz or lower.

Measuring spectrum range: 20 ppm or upper, including between −5 ppm and 15 ppm.

Spinning: off.

Pulse angle: 90°.

^{13}C decoupling: on.

Delay time: Repeating pulse waiting time not less than 60 seconds.

Integrating times: 8 or more times.

Dummy scanning: 2 or more times.

Measuring temperature: A constant temperature between 20°C and 30°C.

System suitability

Test for required detectability: When the procedure is run with the sample solution under the above operating conditions, the SN ratio of the signal of around δ 6.03 ppm is not less than 100.

System performance: When the procedure is run with the sample solution under the above operating conditions, the signal of around δ 6.03 ppm is not overlapped with any signal of obvious foreign substances.

System repeatability: When the test is repeated 6 times with the sample solution under the above operating conditions, the relative standard deviation of the ratio of the resonance intensity A to that of the reference standard for qNMR is not more than 1.0%.

Anti-urokinase serum Antiserum obtained from rabbit immunized with Urokinase, which meets the following performance test. Storage at −20°C or lower.

Performance test—Dissolve 1.0 g of agar in 100 mL of boric acid-sodium hydroxide buffer solution (pH 8.4) by

warming, and pour the solution into a Petri dish to make a depth of about 2 mm. After cooling, bore three of a pair-well 2.5 mm in diameter with a space of 6 mm each other. In one of the wells of each pair-well, place 10 μL of antiurokinase serum, and in each another well, place 10 μL of a solution of Urokinase containing 30,000 Units per mL in isotonic sodium chloride solution, 10 μL of human serum and 10 μL of human urine, respectively, and allow to stand overnight: one or two clear precipitin lines appear between anti-urokinase serum and urokinase, and not appears between anti-urokinase serum and human serum or human urine.

Arbutin for assay $C_{12}H_{16}O_7$ Arbutin for thin-layer chromatography. It meets the requirement of the following arbutin for assay 1 or arbutin for assay 2 (Purity value by quantitative NMR). The former is used after drying (in vacuum, silica gel, 12 hours). The latter is used after allowing to stand in a desiccator, whose humidity is adjusted between 57% and 60% RH at 20 to 25°C with sodium bromide-saturated solution, for 24 hours and weighed at 20 to 25°C under 45 to 60% RH, and corrected for its amount based on the result obtained in the Assay 2.

1) Arbutin for assay 1

Absorbance ⟨*2.24*⟩ $E_{1\,cm}^{1\%}$ (280 nm): 70 – 76 [4 mg, previously dried in a desiccator (in vacuum, silica gel) for 12 hours, water, 100 mL].

Purity Related substances—Dissolve 1 mg of arbutin for assay 1 in 2.5 mL of water, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add water to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, and determine each peak area of the both solutions by the automatic integration method: the total area of the peaks other than arbutin from the sample solution is not larger than the peak area of arbutin from the standard solution.

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 280 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 20°C.

Mobile phase: A mixture of water, methanol and 0.1 mol/L hydrochloric acid TS (94:5:1).

Flow rate: Adjust so that the retention time of arbutin is about 6 minutes.

Time span of measurement: About 3 times as long as the retention time of arbutin, beginning after the solvent peak.

System suitability

Test for required detectability: Pipet 1 mL of the standard solution, and add water to make exactly 20 mL. Confirm that the peak area of arbutin obtained with 10 μL of this solution is equivalent to 3.5 to 6.5% of that with 10 μL of the standard solution.

System performance: Dissolve 1 mg each of arbutin for assay 1, hydroquinone and gallic acid monohydrate in 2 mL of water. When the procedure is run with 10 μL of this solution under the above operating conditions, arbutin, hydroquinone and gallic acid are eluted in this order, and each resolution between these peaks is not less than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of arbutin is not more than 1.5%.

2) Arbutin for assay 2 (Purity value by quantitative NMR)

Unity of peak—Dissolve 1 mg of arbutin for assay 2 in 2.5 mL of water, and use this solution as the sample solution. Perform the test with 10 μL of the sample solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, and compare the absorption spectra of at least 3 points including the top of arbutin peak and around the two middle peak heights of before and after the top: no difference in form is observed between their spectra.

Operating conditions

Detector: A photodiode array detector (wavelength: 280 nm, measuring range of spectrum: 220 – 400 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 20°C.

Mobile phase: A mixture of water, methanol and 0.1 mol/L hydrochloric acid TS (94:5:1).

Flow rate: Adjust so that the retention time of arbutin is about 6 minutes.

System suitability

System performance: Dissolve 1 mg each of arbutin for assay 2, hydroquinone and gallic acid monohydrate in 2 mL of water. When the procedure is run with 10 μL of this solution under the above operating conditions, arbutin, hydroquinone and gallic acid are eluted in this order, and each resolution between these peaks is not less than 1.5, respectively.

Assay—Weigh accurately 1 mg of 1,4-BTMSB-d_4 for nuclear magnetic resonance spectroscopy and 5 mg of arbutin for assay 2, previously allowed to stand in a desiccator, whose humidity is adjusted to 57 to 60% RH at 20 to 25°C with sodium bromide-saturated solution, for 24 hours, at 20 to 25°C under 45 to 60% RH, using an ultramicrobalance, dissolve both in 1 mL of deuterated methanol for nuclear magnetic resonance spectroscopy, and use this solution as the sample solution. Transfer the sample solution into an NMR tube 5 mm in outer diameter, and measure ^{1}H-NMR as directed under Nuclear Magnetic Resonance Spectroscopy ⟨*2.21*⟩ and Crude Drugs Test ⟨*5.01*⟩ according to the following conditions, using 1,4-BTMSB-d_4 for nuclear magnetic resonance spectroscopy as the reference standard

for qNMR. Calculate the resonance intensities, A_1 (equivalent to 2 hydrogen) and A_2 (equivalent to 2 hydrogen), of the signals around δ 6.44 ppm and δ 6.71 ppm assuming the signal of the reference standard for qNMR as δ 0 ppm.

$$\text{Amount (\%) of arbutin } (C_{12}H_{16}O_7) = M_S \times I \times P/(M \times N) \times 1.2020$$

M: Amount (mg) of arbutin for assay 2 taken
M_S: Amount (mg) of 1,4-BTMSB-d_4 for nuclear magnetic resonance spectroscopy taken
I: Sum of the signal resonance intensities, A_1 and A_2, based on the signal resonance intensity of 1,4-BTMSB-d_4 for nuclear magnetic resonance spectroscopy as 18.000
N: Sum of number of the hydrogen derived from A_1 and A_2
P: Purity (%) of 1,4-BTMSB-d_4 for nuclear magnetic resonance spectroscopy

Operating conditions

Apparatus: A nuclear magnetic resonance spectrometer having ^1H resonance frequency of not less than 400 MHz.

Target nucleus: ^1H.

Digital resolution: 0.25 Hz or lower.

Measuring spectrum range: 20 ppm or upper, including between -5 ppm and 15 ppm.

Spinning: off.

Pulse angle: 90°.

^{13}C decoupling: on.

Delay time: Repeating pulse waiting time not less than 60 seconds.

Integrating times: 8 or more times.

Dummy scanning: 2 or more times.

Measuring temperature: A constant temperature between 20°C and 30°C.

System suitability

Test for required detectability: When the procedure is run with the sample solution under the above operating conditions, the SN ratios of the signals of around δ 6.44 ppm and δ 6.71 ppm are not less than 100.

System performance: When the procedure is run with the sample solution under the above operating conditions, the signal of around δ 6.44 ppm and δ 6.71 ppm are not overlapped with any signal of obvious foreign substance, and the ratio of the resonance intensities, A_1/A_2, is between 0.99 and 1.01.

System repeatability: When the test is repeated 6 times with the sample solution under the above operating conditions, the relative standard deviation of the ratio of the resonance intensity, A_1 or A_2, to that of the reference standard for qNMR is not more than 1.0%.

Benzyl parahydroxybenzoate $C_{14}H_{12}O_3$ White, fine crystals or crystalline powder. Freely soluble in ethanol (95), and very slightly soluble in water.

Melting point ⟨*2.60*⟩: 109 – 114°C

Content: not less than 99.0%. Assay—Weigh accurately about 1 g of benzyl parahydroxybenzoate, add exactly 20 mL of 1 mol/L sodium hydroxide VS, heat at about 70°C for 1 hour, and immediately cool in ice. Titrate ⟨*2.50*⟩ the excess sodium hydroxide with 0.5 mol/L sulfuric acid VS up to the second equivalent point (potentiometric titration). Perform a blank determination in the same manner.

$$\text{Each mL of 1 mol/L sodium hydroxide VS} = 228.2 \text{ mg of } C_{14}H_{12}O_3$$

Dehydrocorydaline nitrate for assay $C_{22}H_{24}N_2O_7$ Dehydrocorydaline nitrate for thin-layer chromatography. It meets the requirement of the following dehydrocorydaline nitrate for assay 1 or dehydrocorydaline nitrate for assay 2 (Purity value by quantitative NMR). The former is used after drying in a desiccator (silica gel) for 1 hours, and the latter is used with correction for its amount based on the result obtained in the Assay 2.

1) Dehydrocorydaline nitrate for assay 1

Absorbance ⟨*2.24*⟩ $E^{1\%}_{1\,cm}$ (333 nm): 577 – 642 (3 mg, water, 500 mL). Use the sample dried in a desiccator (silica gel) for not less than 1 hour for the test.

Purity Related substances—Dissolve 5.0 mg of dehydrocorydaline nitrate for assay 1 in 10 mL of the mobile phase, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add the mobile phase to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 5 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, and determine each peak area by the automatic integration method: the total area of the peaks other than dehydrocorydaline obtained from the sample solution is not larger than the peak area of dehydrocorydaline from the standard solution.

Operating conditions

Column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay under Corydalis Tuber.

Detector: An ultraviolet absorption photometer (wavelength: 230 nm).

Time span of measurement: About 3 times as long as the retention time of dehydrocorydaline, beginning after the peak of nitric acid.

System suitability

Test for required detectability: Pipet 1 mL of the standard solution, and add the mobile phase to make exactly 20 mL. Confirm that the peak area of dehydrocorydaline obtained with 5 μL of this solution is equivalent to 3.5 to 6.5% of that with 5 μL of the standard solution.

System performance: Dissolve 1 mg each of dehydrocorydaline nitrate for assay 1 and berberine chloride hydrate in 20 mL of a mixture of water and acetonitrile (20:9). When the procedure is run with 5 μL of this solution under the above operating conditions, berberine and dehydrocorydaline are eluted in this order with the resolution between these peaks being not less than 1.5.

System repeatability: When the test is repeated 6 times with 5 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of dehydrocorydaline is not more than 1.5%.

2) Dehydrocorydaline nitrate for assay 2 (Purity value by quantitative NMR)

Unity of peak—Dissolve 1 mg of dehydrocorydaline nitrate for assay 2 in 2 mL of a mixture of methanol and dilute hydrochloric acid (3:1), and use this solution as the sample solution. Perform the test with 5 μL of the sample solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and compare the absorption spectra of at least 3 points including the top of dehydrocorydaline peak and around the two middle peak heights of before and after the top: no difference in form is observed among their spectra.

Operating conditions

Column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay under Corydalis Tuber.

Detector: A photodiode array detector (wavelength: 230 nm, spectrum range of measurement: 220 – 400 nm).

System suitability

System performance: Dissolve 1 mg each of dehydrocorydaline nitrate for assay 2 and berberine chloride hydrate in 20 mL of a mixture of water and acetonitrile (20:9). When the procedure is run with 5 μL of this solution under the above operating conditions, berberine and dehydrocorydaline are eluted in this order with the resolution between these peaks being not less than 1.5.

Assay—Weigh accurately 5 mg of dehydrocorydaline nitrate for assay 2 and 1 mg of DDS-d_6 for nuclear magnetic resonance spectroscopy using an ultramicrobalance, dissolve both in 1 mL of deuterated dimethylsulfoxide for nuclear magnetic resonance spectroscopy, and use this solution as the sample solution. Transfer the sample solution into an NMR tube 5 mm in outer diameter, and measure ^{1}H-NMR as directed under Nuclear Magnetic Resonance Spectroscopy <*2.21*> and Crude Drugs Test <*5.01*> according to the following conditions, using DDS-d_6 for nuclear magnetic resonance spectroscopy as the reference standard for qNMR. Calculate the resonance intensity, A (equivalent to 1 hydrogen) of the signal around δ 7.42 ppm assuming the signal of the reference standard for qNMR as δ 0 ppm.

Amount (%) of dehydrocorydaline nitrate ($C_{22}H_{24}N_2O_7$)
$= M_S \times I \times P/(M \times N) \times 1.9096$

M: Amount (mg) of dehydrocorydaline nitrate for assay 2 taken
M_S: Amount (mg) of DDS-d_6 for nuclear magnetic resonance spectroscopy taken
I: Signal resonance intensity A based on the signal resonance intensity of DDS-d_6 for nuclear magnetic resonance spectroscopy as 9.000
N: Number of the hydrogen derived from A
P: Purity (%) of DDS-d_6 for nuclear magnetic resonance spectroscopy

Operating conditions

Apparatus: A nuclear magnetic resonance spectrometer having ^{1}H resonance frequency of not less than 400 MHz.

Target nucleus: ^{1}H.

Digital resolution: 0.25 Hz or lower.

Measuring spectrum range: 20 ppm or upper, including between −5 ppm and 15 ppm.

Spinning: off.

Pulse angle: 90°.

^{13}C decoupling: on.

Delay time: Repeating pulse waiting time not less than 60 seconds.

Integrating times: 8 or more times.

Dummy scanning: 2 or more times.

Measuring temperature: A constant temperature between 20°C and 30°C.

System suitability

Test for required detectability: When the procedure is run with the sample solution under the above operating conditions, the SN ratio of the signal around δ 7.42 ppm is not less than 100.

System performance: When the procedure is run with the sample solution under the above operating conditions, the signal around δ 7.42 ppm is not overlapped with any signal of obvious foreign substances.

System repeatability: When the test is repeated 6 times with the sample solution under the above operating conditions, the relative standard deviation of the ratio of the resonance intensity A to that of the reference standard for qNMR is not more than 1.0%.

Dehydrocorydaline nitrate for thin-layer chromatography $C_{22}H_{24}N_2O_7$ Yellow, crystals or crystalline powder. Sparingly soluble in methanol, and slightly soluble in water and in ethanol (99.5). Melting point: about 240°C (with decomposition).

Purity Related substances—Dissolve 5.0 mg of dehydrocorydaline nitrate for thin-layer chromatography in 1 mL of a mixture of water and methanol (1:1), and use this solution as the sample solution. Pipet 0.5 mL of the sample solution, add a mixture of water and methanol (1:1) to make exactly 50 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop immediately with a mixture of methanol, a solution of ammonium acetate (3 in 10) and acetic acid (100) (20:1:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly Dragendorff's TS on the plate, air-dry the plate, and then spray evenly sodium nitrite TS: the spot other than the principal spot obtained from the sample solution is not more intense than the spot from the standard solution.

Diphenyl sulfone for assay $C_{12}H_{10}O_2S$ White, crystals or crystalline powder. It dissolves in dimethylsulfoxide.

It is used after correcting with the amount of diphenyl sulfone obtained in the Assay.

Identification—Proceed as directed in the Assay: it exhibits a triplet-like signal equivalent to 4 protons around δ 7.65 ppm, a triplet-like signal equivalent to 2 protons around δ 7.73 ppm, and a doublet-like signal equivalent to 4 protons around δ 7.99 ppm.

Unity of peak—Dissolve 10 mg of diphenyl sulfone for assay in 100 mL of methanol. To 10 mL of this solution add methanol to make 100 mL, and use this solution as the sample solution. Perform the test with 10 μL of the sample solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and compare the absorption spectra of at least 3 points including the top of diphenyl sulfone peak and around the two middle peak heights of before and after the top: no difference in form is observed between their spectra.

Operating conditions

Column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay under Perilla Herb.

Detector: A photodiode array detector (wavelength: 234 nm, spectrum range of measurement: 220 – 400 nm).

System suitability

System performance: Dissolve 1 mg each of (*E*)-asarone and perillaldehyde for thin-layer chromatography in the sample solution to make 50 mL. When the procedure is run with 10 μL of this solution under the above operating conditions, diphenyl sulfone, perillaldehyde and (*E*)-asarone are eluted in this order with the resolutions between these peaks being not less than 1.5.

The unity of peak is unnecessary if the content (%) of diphenyl sulfone ($C_{12}H_{10}O_2S$) is between 99.5% and 100.5%.

Assay—Weigh accurately 5 mg of diphenyl sulfone for assay and 1 mg of DSS-d_6 for nuclear magnetic resonance spectroscopy using an ultramicrobalance, dissolve in 2 mL of deuterated dimethylsulfoxide for nuclear magnetic resonance spectroscopy, and use this solution as the sample solution. Transfer the sample solution into an NMR tube 5 mm in outer diameter, measure ^{1}H-NMR as directed under Nuclear Magnetic Resonance Spectroscopy <*2.21*> and Crude Drugs Test <*5.01*> according to the following conditions, using DSS-d_6 for nuclear magnetic resonance spectroscopy as the reference standard for qNMR. Calculate the resonance intensities, A_1 (equivalent to 6 hydrogens) and A_2 (equivalent to 4 hydrogens), of the signals around δ 7.64 – 7.74 ppm and δ 7.98 – 8.01 ppm assuming the signal of the reference standard for qNMR as δ 0 ppm.

$$\text{Amount (\%) of diphenyl sulfone } (C_{12}H_{10}O_2S) = M_S \times I \times P/(M \times N) \times 0.9729$$

M: Amount (mg) of diphenyl sulfone for assay taken

M_S: Amount (mg) of DSS-d_6 for nuclear magnetic resonance spectroscopy taken

I: Sum of the signal resonance intensities, A_1 and A_2, based on the signal resonance intensity of DSS-d_6 for nuclear magnetic resonance spectroscopy as 9.000

N: Sum of numbers of the hydrogen derived from A_1 and A_2

P: Purity (%) of DSS-d_6 for nuclear magnetic resonance spectroscopy

Operating conditions

Apparatus: An apparatus of nuclear magnetic resonance spectrum measurement having ^{1}H resonance frequency of not less than 400 MHz.

Target nucleus: ^{1}H.

Digital resolution: 0.25 Hz or lower.

Measuring spectrum range: 20 ppm or upper, including between −5 ppm and 15 ppm.

Spinning: off.

Pulse angle: 90°.

^{13}C decoupling: on.

Delay time: Repeating pulse waiting time not less than 60 seconds.

Integrating times: 8 or more times.

Dummy scanning: 2 or more times.

Measuring temperature: A constant temperature between 20°C and 30°C.

System suitability

Test for required detectability: When the procedure is run with the sample solution under the above operating conditions, the SN ratio of each signal around δ 7.64 – 7.74 ppm and δ 7.98 – 8.01 ppm is not less than 100.

System performance: When the procedure is run with the sample solution under the above operating conditions, the signals around δ 7.64 – 7.74 ppm and δ 7.98 – 8.01 ppm are not overlapped with any signal of obvious foreign substance, and the ratio of the resonance intensities, $(A_1/6)/(A_2/4)$, of each signal around δ 7.64 – 7.74 ppm and δ 7.98 – 8.01 ppm is between 0.99 and 1.01, respectively.

System repeatability: When the test is repeated 6 times with the sample solution under the above operating conditions, the relative standard deviation of the ratio of the resonance intensity, A_1 or A_2, to that of the reference standard for qNMR are not more than 1.0%.

[6]-Gingerol for assay $C_{17}H_{26}O_4$ [6]-Gingerol for thin-layer chromatography. It meets the following additional requirements. It is used with correction for its amount based on the result obtained in the Assay.

Unity of peak—Dissolve 5 mg of [6]-gingerol for assay in 5 mL of methanol, and use this solution as the sample solution. Perform the test with 10 μL of the sample solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and compare the absorption spectra of at least 3 points including the top of [6]-gingerol peak and around the two middle peak heights of before and after the top: no difference in form is observed among their spectra.

Operating conditions

Column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay (3) under Hangekobokuto Extract.

Detector: A photodiode array detector (wavelength: 282 nm, measuring range of spectrum: 220 – 400 nm).

System suitability

System performance: When the procedure is run with 10 μL of the sample solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of [6]-gingerol are not less than 5000 and not more than 1.5, respectively.

Assay—Weigh accurately 5 mg of [6]-gingerol for assay and 1 mg of 1,4-BTMSB-d_4 for nuclear magnetic resonance spectroscopy using an ultramicrobalance, dissolve both in 1 mL of deuterated methanol for nuclear magnetic resonance spectroscopy, and use this solution as the sample solution. Transfer the sample solution into an NMR tube 5 mm in outer diameter, and measure ^{1}H-NMR as directed under Nuclear Magnetic Resonance Spectroscopy <*2.21*> and Crude Drugs Test <*5.01*> according to the following conditions, using 1,4-BTMSB-d_4 for nuclear magnetic resonance spectroscopy as the reference standard for qNMR. Calculate the resonance intensities, A_1 (equivalent to 3 hydrogens) and A_2 (equivalent to 1 hydrogen), of the signals around δ 3.56 ppm and δ 6.52 ppm assuming the signal of the reference standard for qNMR as δ 0 ppm.

$$\text{Amount (\%) of [6]-gingerol } (C_{17}H_{26}O_4) = M_S \times I \times P/(M \times N) \times 1.2997$$

M: Amount (mg) of [6]-gingerol for assay taken
M_S: Amount (mg) of 1,4-BTMSB-d_4 for nuclear magnetic resonance spectroscopy taken
I: Sum of the signal resonance intensities, A_1 and A_2, based on the signal resonance intensity of 1,4-BTMSB-d_4 for nuclear magnetic resonance spectroscopy as 18.000
N: Sum of the numbers of the hydrogen derived from A_1 and A_2
P: Purity (%) of 1,4-BTMSB-d_4 for nuclear magnetic resonance spectroscopy

Operating conditions

Apparatus: A nuclear magnetic resonance spectrometer having ^{1}H resonance frequency of not less than 400 MHz.

Target nucleus: ^{1}H.

Digital resolution: 0.25 Hz or lower.

Measuring spectrum range: 20 ppm or upper, including between −5 ppm and 15 ppm.

Spinning: off.

Pulse angle: 90°.

^{13}C decoupling: on.

Delay time: Repeating pulse waiting time not less than 60 seconds.

Integrating times: 8 or more times.

Dummy scanning: 2 or more times.

Measuring temperature: A constant temperature between 20°C and 30°C.

System suitability

Test for required detectability: When the procedure is run with the sample solution under the above operating conditions, the SN ratio of each signal around δ 3.56 ppm and δ 6.52 ppm is not less than 100.

System performance: When the procedure is run with the sample solution under the above operating conditions, the signals around δ 3.56 ppm and δ 6.52 ppm are not overlapped with any signal of obvious foreign substance, and the ratio of the resonance intensities, $(A_1/3)/A_2$, of each signal around δ 3.56 ppm and δ 6.52 ppm is between 0.99 and 1.01.

System repeatability: When the test is repeated 6 times with the sample solution under the above operating conditions, the relative standard deviation of the ratio of the resonance intensity, A_1 or A_2, to that of the reference standard for qNMR is not more than 1.0%.

Hirsutine for assay $C_{22}H_{28}N_2O_3$ Hirsutine for thin-layer chromatography. It meets the requirement of the following hirsutine for assay 1 or hirsutine for assay 2 (Purity value by quantitative NMR). Hirsutine for assay 2 is used with correction for its amount based on the result obtained in the Assay 2.

1) Hirsutine for assay 1

Absorbance <*2.24*>: $E^{1\%}_{1\,cm}$ (245 nm): 354 – 389 (5 mg calculated on the anhydrous basis, a mixture of methanol and dilute acetic acid (7:3), 500 mL).

Purity Related substances—Dissolve 5 mg of hirsutine for assay 1 in 100 mL of a mixture of methanol and dilute acetic acid (7:3), use this solution as the sample solution. Pipet 1 mL of the sample solution, add a mixture of methanol and dilute acetic acid (7:3) to make exactly 50 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid-chromatography <*2.01*> according to the following conditions. Determine each peak area by the automatic integration method: the total area of the peaks other than hirsutine obtained from the sample solution is not larger than the peak area of hirsutine from the standard solution.

Operating conditions

Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay under Uncaria Hook.

Time span of measurement: About 1.5 times as long as the retention time of hirsutine, beginning after the solvent peak.

System suitability

Test for required detectability: Pipet 1 mL of the standard solution, add a mixture of methanol and dilute acetic acid (7:3) to make exactly 20 mL. Confirm that the peak area of hirsutine obtained with 20 μL of this solution is equivalent to 3.5 to 6.5% of that with 20 μL of the standard solution.

System performance: Dissolve 1 mg of rhynchophylline for assay in 20 mL of a mixture of methanol and dilute acetic acid (7:3). To 5 mL of this solution add 1 mL of ammonia solution (28), and warm at about 50°C for 2 hours or heat under a reflux condenser for 10 minutes. After cooling, to 1 mL of the reaction solution add a mixture of methanol and dilute acetic acid (7:3) to make 5 mL. When the procedure is run with 20 μL of this solution under the above operating conditions, the peak of isorhynchophylline is observed in addition to the peak of rhynchophylline, and the resolution between these peaks is not less than 1.5.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of hirsutine is not more than 1.5%.

2) Hirsutine for assay 2 (Purity value by quantitative NMR)

Unity of peak—Dissolve 1 mg of hirsutine for assay 2 in 20 mL of a mixture of methanol and dilute acetic acid (7:3), and use this solution as the sample solution. Perform the test with 20 μL of the sample solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and compare the absorption spectra of at least 3 points including the top of hirsutine peak and around the two middle peak heights of before and after the top: no difference in form is observed among their spectra.

Operating conditions

Column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay under Uncaria Hook.

Detector: A photodiode array detector (wavelength: 245 nm, spectrum range of measurement: 220 – 400 nm).

System suitability

System performance: Dissolve 1 mg of rhynchophylline for assay in 20 mL of a mixture of methanol and dilute acetic acid (7:3). To 5 mL of this solution add 1 mL of ammonia solution (28), and warm at about 50°C for 2 hours or heat under a reflux condenser for 10 minutes. After cooling, to 1 mL of the reaction solution add a mixture of methanol and dilute acetic acid (7:3) to make 5 mL. When the procedure is run with 20 μL of this solution under the above operating conditions, the peak of isorhynchophylline is observed in addition to the peak of rhynchophylline, and the resolution between these peaks is not less than 1.5.

Assay—Weigh accurately 5 mg of hirsutine for assay 2 and 1 mg of 1,4-BTMSB-d_4 for nuclear magnetic resonance spectroscopy using an ultramicrobalance, dissolve both in 1 mL of deuterated acetone for nuclear magnetic resonance spectroscopy, and use this solution as the sample solution. Transfer the sample solution into an NMR tube 5 mm in outer diameter, and measure ^{1}H-NMR as directed under Nuclear Magnetic Resonance Spectroscopy <*2.21*> and Crude Drugs Test <*5.01*> according to the following conditions, using 1,4-BTMSB-d_4 for nuclear magnetic resonance spectroscopy as the reference standard for qNMR. Calculate the resonance intensity A (equivalent to 2 hydrogens) of the signal around δ 6.70 – δ 6.79 ppm assuming the signal of the reference standard for qNMR as δ 0 ppm.

$$\text{Amount (\%) of hirsutine } (C_{22}H_{28}N_2O_3) = M_S \times I \times P/(M \times N) \times 1.6268$$

M: Amount (mg) of hirsutine for assay 2 taken
M_S: Amount (mg) of 1,4-BTMSB-d_4 for nuclear magnetic resonance spectroscopy taken
I: Signal resonance intensity A based on the signal resonance intensity of 1,4-BTMSB-d_4 for nuclear magnetic resonance spectroscopy as 18.000
N: Number of the hydrogen derived from A
P: Purity (%) of 1,4-BTMSB-d_4 for nuclear magnetic resonance spectroscopy

Operating conditions

Apparatus: A nuclear magnetic resonance spectrometer having ^{1}H resonance frequency of not less than 400 MHz.

Target nucleus: ^{1}H.

Digital resolution: 0.25 Hz or lower.

Measuring spectrum range: 20 ppm or upper, including between −5 ppm and 15 ppm.

Spinning: off.

Pulse angle: 90°.

^{13}C decoupling: on.

Delay time: Repeating pulse waiting time not less than 60 seconds.

Integrating times: 8 or more times.

Dummy scanning: 2 or more times.

Measuring temperature: A constant temperature between 20°C and 30°C.

System suitability

Test for required detectability: When the procedure is run with the sample solution under the above operating conditions, the SN ratio of the signal around δ 6.70 – δ 6.79 ppm is not less than 100.

System performance: When the procedure is run with the sample solution under the above operating conditions, the signal around δ 6.70 – δ 6.79 ppm is not overlapped with any signal of obvious foreign substances.

System repeatability: When the test is repeated 6 times with the sample solution under the above operating conditions, the relative standard deviation of the ratio of the resonance intensity A to that of the reference standard for qNMR is not more than 1.0%.

Loganin for assay $C_{17}H_{26}O_{10}$ Loganin for thin-layer chromatography. It meets the following additional requirements. It is used with correction for its amount based on the result obtained in the Assay.

Unity of peak—Dissolve 2 mg of loganin for assay in 5 mL of the mobile phase, and use this solution as the sample solution. Perform the test with 10 μL of the sample solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and compare the absorption spectra of at least 3 points including the top of loganin peak and around the two middle peak heights of before and after the top: no difference in form is observed among their spectra.

Operating conditions

Column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay (1) under Goshajinkigan Extract.

Detector: A photodiode array detector (wavelength: 238 nm, spectrum range of measurement: 220 – 400 nm).

System suitability

System performance: When the procedure is run with 10 μL of the sample solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of loganin are not less than 5000 and not more than 1.5, respectively.

Assay—Weigh accurately 5 mg of loganin for assay and 1 mg of 1,4-BTMSB-d_4 for nuclear magnetic resonance spectroscopy using an ultramicrobalance, dissolve in 1 mL of deuterated methanol for nuclear magnetic resonance spec-

troscopy, and use this solution as the sample solution. Transfer the sample solution into an NMR tube 5 mm in outer diameter, and measure ^{1}H-NMR as directed under Nuclear Magnetic Resonance Spectroscopy <*2.21*> and Crude Drugs Test <*5.01*> according to the following conditions, using 1,4-BTMSB-d_4 for nuclear magnetic resonance spectroscopy as the reference standard for qNMR. Calculate the resonance intensity A (equivalent to 1 hydrogen) of the signal around δ 7.14 ppm assuming the signal of the reference standard for qNMR as δ 0 ppm.

$$\text{Amount (\%) of loganin } (C_{17}H_{26}O_{10}) = M_S \times I \times P/(M \times N) \times 1.7235$$

M: Amount (mg) of loganin for assay taken
M_S: Amount (mg) of 1,4-BTMSB-d_4 for nuclear magnetic resonance spectroscopy taken
I: Signal resonance intensity A based on the signal resonance intensity of 1,4-BTMSB-d_4 for nuclear magnetic resonance spectroscopy as 18.000
N: Number of the hydrogen derived from A
P: Purity (%) of 1,4-BTMSB-d_4 for nuclear magnetic resonance spectroscopy

Operating conditions

Apparatus: A nuclear magnetic resonance spectrometer having ^{1}H resonance frequency of not less than 400 MHz.

Target nucleus: ^{1}H.

Digital resolution: 0.25 Hz or lower.

Measuring spectrum range: 20 ppm or upper, including between −5 ppm and 15 ppm.

Spinning: off.

Pulse angle: 90°.

^{13}C decoupling: on.

Delay time: Repeating pulse waiting time not less than 60 seconds.

Integrating times: 8 or more times.

Dummy scanning: 2 or more times.

Measuring temperature: A constant temperature between 20°C and 30°C.

System suitability

Test for required detectability: When the procedure is run with the sample solution under the above operating conditions, the SN ratio of each signal around δ 5.02 ppm and δ 7.14 ppm is not less than 100.

System performance: When the procedure is run with the sample solution under the above operating conditions, the two signals around δ 5.02 ppm and δ 7.14 ppm are not overlapped with any signal of obvious foreign substance. Furthermore, when determined the resonance intensities A_1 and A, both equivalent to 1 hydrogen, of each signal around δ 5.02 ppm and δ 7.14 ppm, the ratio of them, A_1/A, is between 0.99 and 1.01.

System repeatability: When the test is repeated 6 times with the sample solution under the above operating conditions, the relative standard deviation of the ratio of the resonance intensity A to that of the reference standard for qNMR is not more than 1.0%.

Plantago seed for thin-layer chromatography [Same as the monograph Plantago Seed meeting the following additional specifications.]

Identification (1) To 1 g of pulverized plantago seed for thin-layer chromatography add 3 mL of methanol, and warm on a water bath for 3 minutes. After cooling, centrifuge, and use the supernatant liquid as the sample solution. Perform the test with the sample solution as directed under Thin-layer Chromatography <*2.03*>. Spot 10 μL of the sample solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of acetone, ethyl acetate, water and acetic acid (100) (10:10:3:1) to a distance of about 10 cm, and air-dry the plate. Splay evenly 4-methoxybenzaldehyde-sulfuric acid TS on the plate, heat the plate at 105°C for 10 minutes: spots equivalent to those described below appear.

*R*f value	Color and shape of the spot
Around 0	A strong spot, very dark blue
Around 0.08	A very dark blue spot
Around 0.1 – 0.2	A leading spot, very dark blue
Around 0.25	A strong spot, deep blue (corresponding to plantagoguanidinic acid)
Around 0.35	A strong spot, dark grayish blue (corresponding to geniposidic acid)
Around 0.45	A weak spot, grayish yellowish green
Around 0.50	A strong spot, deep yellow-green (corresponding to verbascoside)
Around 0.6	A weak spot, light blue
Around 0.85	A deep blue spot
Around 0.9 – 0.95	A tailing spot, grayish blue

(2) Proceed with the sample solution obtained in (1) as directed in the method under (1), except using a mixture of ethyl acetate, water and formic acid (6:1:1) as developing solvent: spots equivalent to those described below appear.

*R*f value	Color and shape of the spot
Around 0	A yellow-greenish dark gray spot
Around 0.05	A weak spot, dark grayish yellow-green
Around 0.2	A weak spot, dark green
Around 0.25	A strong spot, dark reddish purple (corresponding to geniposidic acid)
Around 0.35	A weak spot, bright blue
Around 0.4 – 0.45	A weak tailing spot, dull greenish blue
Around 0.45	A strong spot, deep yellow-green (corresponding to verbascoside)
Around 0.5	A strong spot, deep blue (corresponding to plantagoguanidinic acid)
Around 0.95	A strong spot, dark grayish blue-green
Around 0.97	A dark grayish blue-green spot

Rhynchophylline for assay $C_{22}H_{28}N_2O_4$ Rhynchophylline for thin-layer chromatography. It meets the require-

ment of the following rhynchophylline for assay 1 or rhynchophylline for assay 2 (Purity value by quantitative NMR). Rhynchophylline for assay 2 is used with correction for its amount based on the result obtained in the Assay 2.

1) Rhynchophylline for assay 1

Absorbance ⟨*2.24*⟩: $E^{1\%}_{1\,cm}$ (245 nm): 473 – 502 (5 mg, a mixture of methanol and dilute acetic acid (7:3), 500 mL).

Purity Related substances—Dissolve 5 mg of rhynchophylline for assay 1 in 100 mL of a mixture of methanol and dilute acetic acid (7:3), and use this solution as the sample solution. Pipet 1 mL of the sample solution, add a mixture of methanol and dilute acetic acid (7:3) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions. Determine the peak area of each solution by the automatic integration method: the total area of the peaks other than rhynchophylline obtained from the sample solution is not larger than the peak area of rhynchophylline from the standard solution.

Operating conditions

Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay under Uncaria Hook.

Time span of measurement: About 4 times as long as the retention time of rhynchophylline, beginning after the solvent peak.

System suitability

Test for required detectability: Pipet 1 mL of the standard solution, add a mixture of methanol and dilute acetic acid (7:3) to make exactly 20 mL. Confirm that the peak area of rhynchophylline obtained with 20 μL of this solution is equivalent to 3.5 to 6.5% of that with 20 μL of the standard solution.

System performance: To 5 mL of the sample solution add 1 mL of ammonia solution (28), and warm at about 50°C for 2 hours or heat under a reflux condenser for 10 minutes. After cooling, to 1 mL of the reaction solution add a mixture of methanol and dilute acetic acid (7:3) to make 5 mL. When the procedure is run with 20 μL of this solution under the above operating conditions, the peak of isorhynchophylline is observed in addition to the peak of rhynchophylline, and the resolution between these peaks is not less than 1.5.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of rhynchophylline is not more than 1.5%.

2) Rhynchophylline for assay 2 (Purity value by quantitative NMR)

Unity of peak—Dissolve 1 mg of rhynchophylline for assay 2 in 100 mL of a mixture of methanol and dilute acetic acid (7:3), and use this solution as the sample solution. Perform the test with 20 μL of the sample solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, and compare the absorption spectra of at least 3 points including the top of rhynchophylline peak and around the two middle peak heights of before and after the top: no difference in form is observed among their spectra.

Operating conditions

Column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay under Uncaria Hook.

Detector: A photodiode array detector (wavelength: 245 nm, spectrum range of measurement: 220 – 400 nm).

System suitability

System performance: Dissolve 1 mg of rhynchophylline for assay 2 in 20 mL of a mixture of methanol and dilute acetic acid (7:3). To 5 mL of this solution add 1 mL of ammonia solution (28), and warm at about 50°C for 2 hours or heat under a reflux condenser for 10 minutes. After cooling, to 1 mL of the reaction solution add a mixture of methanol and dilute acetic acid (7:3) to make 5 mL. When the procedure is run with 20 μL of this solution under the above operating conditions, the peak of isorhynchophylline is observed in addition to the peak of rhynchophylline, and the resolution between these peaks is not less than 1.5.

Assay—Weigh accurately 5 mg of rhynchophylline for assay 2 and 1 mg of 1,4-BTMSB-d_4 for nuclear magnetic resonance spectroscopy using an ultramicrobalance, dissolve both in 1 mL of deuterated acetone for nuclear magnetic resonance spectroscopy, and use this solution as the sample solution. Transfer the sample solution into an NMR tube 5 mm in outer diameter, and measure ^{1}H-NMR as directed under Nuclear Magnetic Resonance Spectroscopy ⟨*2.21*⟩ and Crude Drugs Test ⟨*5.01*⟩ according to the following conditions, using 1,4-BTMSB-d_4 for nuclear magnetic resonance spectroscopy as the reference standard for qNMR. Calculate the resonance intensities, A_1 (equivalent to 1 hydrogen) and A_2 (equivalent to 1 hydrogen), of the signals around δ 6.60 ppm and δ 6.73 ppm assuming the signal of the reference standardfor qNMR as δ 0 ppm.

$$\text{Amount (\%) of rhynchophylline } (C_{22}H_{28}N_2O_4) = M_S \times I \times P/(M \times N) \times 1.6974$$

M: Amount (mg) of rhynchophylline for assay 2 taken

M_S: Amount (mg) of 1,4-BTMSB-d_4 for nuclear magnetic resonance spectroscopy taken

I: Sum of the signal resonance intensities, A_1 and A_2, based on the signal resonance intensity of 1,4-BTMSB-d_4 for nuclear magnetic resonance spectroscopy as 18.000

N: Sum of the numbers of the hydrogen derived from A_1 and A_2

P: Purity (%) of 1,4-BTMSB-d_4 for nuclear magnetic resonance spectroscopy

Operating conditions

Apparatus: A nuclear magnetic resonance spectrometer having ^{1}H resonance frequency of not less than 400 MHz.

Target nucleus: ^{1}H.

Digital resolution: 0.25 Hz or lower.

Measuring spectrum range: 20 ppm or upper, including between −5 ppm and 15 ppm.

Spinning: off.

Pulse angle: 90°.

^{13}C decoupling: on.

Delay time: Repeating pulse waiting time not less than 60 seconds.

Integrating times: 8 or more times.

Dummy scanning: 2 or more times.

Measuring temperature: A constant temperature between 20°C and 30°C.

System suitability

Test for required detectability: When the procedure is run with the sample solution under the above operating conditions, the SN ratio of the signal around δ 6.60 ppm and δ 6.73 ppm is not less than 100.

System performance: When the procedure is run with the sample solution under the above operating conditions, the signals around δ 6.60 ppm and δ 6.73 ppm are not overlapped with any signal of obvious foreign substances, and the ratio of the resonance intensities, A_1/A_2, of the signals around δ 6.60 ppm and δ 6.73 ppm is between 0.99 and 1.01.

System repeatability: When the test is repeated 6 times with the sample solution under the above operating conditions, the relative standard deviation of the ratios of the resonance intensities, A_1 and A_2, to that of the reference standard for qNMR are not more than 1.0%.

[6]-Shogaol for assay $C_{17}H_{24}O_3$ [6]-Shogaol for thin-layer chromatography. It meets the following additional requirements . It is used with correction for its amount based on the result obtained in the Assay.

Unity of peak—Dissolve 5 mg of [6]-shogaol for assay in 10 mL of a mixture of acetonitrile and water (2:1), and use this solution as the sample solution. Perform the test with 10 μL of the sample solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and compare the absorption spectra of at least 3 points including the top of [6]-shogaol peak and around the two middle peak heights of before and after the top: no difference in form is observed among their spectra.

Operating conditions

Column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay (2) under Mukoi-Daikenchuto Extract.

Detector: A photodiode array detector (wavelength: 225 nm, spectrum range of measurement: 220 – 400 nm).

System suitability

System performance: When the procedure is run with 10 μL of the sample solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of [6]-shogaol are not less than 5000 and not more than 1.5, respectively.

Assay—Weigh accurately 5 mg of [6]-shogaol for assay and 1 mg of 1,4-BTMSB-d_4 for nuclear magnetic resonance spectroscopy using an ultramicrobalance, dissolve in 1 mL of deuterated methanol for nuclear magnetic resonance spectroscopy, and use this solution as the sample solution. Transfer the sample solution into an NMR tube 5 mm in outer diameter, and measure ^1H-NMR as directed under Nuclear Magnetic Resonance Spectroscopy <*2.21*> and Crude Drugs Test <*5.01*> according to the following conditions, using 1,4-BTMSB-d_4 for nuclear magnetic resonance spectroscopy as the reference standard for qNMR. Calculate the resonance intensity A (equivalent to 3 hydrogens) of the signal around δ 3.57 ppm assuming the signal of the reference standard for qNMR as δ 0 ppm.

$$\text{Amount (\%) of [6]-shogaol } (C_{17}H_{24}O_3) = M_S \times I \times P/(M \times N) \times 1.2202$$

M: Amount (mg) of [6]-shogaol for assay taken

M_S: Amount (mg) of 1,4-BTMSB-d_4 for nuclear magnetic resonance spectroscopy taken

I: Signal resonance intensity A based on the signal resonance intensity of 1,4-BTMSB-d_4 for nuclear magnetic resonance spectroscopy as 18.000

N: Number of the hydrogen derived from A

P: Purity (%) of 1,4-BTMSB-d_4 for nuclear magnetic resonance spectroscopy

Operating conditions

Apparatus: A nuclear magnetic resonance spectrometer having ^1H resonance frequency of not less than 400 MHz.

Target nucleus: ^1H.

Digital resolution: 0.25 Hz or lower.

Measuring spectrum range: 20 ppm or upper, including between −5 ppm and 15 ppm.

Spinning: off.

Pulse angle: 90°.

^{13}C decoupling: on.

Delay time: Repeating pulse waiting time not less than 60 seconds.

Integrating times: 8 or more times.

Dummy scanning: 2 or more times.

Measuring temperature: A constant temperature between 20°C and 30°C.

System suitability

Test for required detectability: When the procedure is run with the sample solution under the above operating conditions, the SN ratio of each signal around δ 3.57 ppm and δ 6.37 – 6.43 ppm is not less than 100.

System performance: When the procedure is run with the sample solution under the above operating conditions, the two signals around δ 3.57 ppm and δ 6.37 – 6.43 ppm are not overlapped with any signal of obvious foreign substance. Furthermore, when determined the resonance intensities, A (equivalent to 3 hydrogens) and A_1 (equivalent to 2 hydrogens) of each signal around δ 3.57 ppm and δ 6.37 – 6.43 ppm, the ratio of the resonance intensities, $(A/3)/(A_1/2)$, of each signal around δ 3.57 ppm and δ 6.37 – 6.43 ppm is between 0.99 and 1.01.

System repeatability: When the test is repeated 6 times with the sample solution under the above operating conditions, the relative standard deviation of the ratio of the resonance intensity A to that of the reference standard for qNMR is not more than 1.0%.

Sindbis virus RNA virus of *Togaviridae*, proliferated by chick embryo cell primary culture or chick embryo fibrob-

last-derived cell line (ATCC CRL-12203, etc.) culture. Determine the number of plaques on the cell culture, and use the virus with not less than 1×10^8 PFU/mL.

Delete the following:

Blocking TS for nartograstim test

Bovine serum albumin TS for nartograstim test

Buffer solution for nartograstim sample

Freund's complete adjuvant

Molecular mass marker for nartograstim test

Polyacrylamide gel for nartograstim

Potency measuring medium for nartograstim test

Rabbit anti-nartograstim antibody

Rabbit anti-nartograstim antibody TS

Reduction buffer solution for nartograstim sample

Subculture medium for nartograstim

Washing fluid for nartograstim test

9.42 Solid Supports/Column Packings for Chromatography

Add the following:

Octadecylsilyl and octylsilyl groups bound to porous silica gel for liquid chromatography A porous silica gel bound with octadecylsilyl and octylsilyl groups, prepared for liquid chromatography.

Polyamine silica gel for liquid chromatography Prepared for liquid chromatography.

Official Monographs

Remove the following items from the Purity and move up the after item number sequentially:

Official Monographs	Purity Tests to be removed
Acebutolol Hydrochloride	Heavy metals, Arsenic
Acemetacin	Heavy metals
Acetaminophen	Heavy metals, Arsenic
Acetazolamide	Heavy metals
Acetic Acid	Heavy metals
Glacial Acetic Acid	Heavy metals
Acetohexamide	Heavy metals
Acetylcholine Chloride for Injection	Heavy metals
Acetylcysteine	Heavy metals
Aciclovir	Heavy metals
Aclarubicin Hydrochloride	Heavy metals
Acrinol Hydrate	Heavy metals
Adrenaline	Heavy metals
Afloqualone	Heavy metals
Alacepril	Heavy metals
L-Alanine	Heavy metals
Aldioxa	Heavy metals
Alendronate Sodium Hydrate	Heavy metals
Alimemazine Tartrate	Heavy metals, Arsenic
Allopurinol	Heavy metals, Arsenic
Alprazolam	Heavy metals
Alprenolol Hydrochloride	Heavy metals, Arsenic
Alprostadil Injection	Heavy metals
Dried Aluminum Hydroxide Gel	Heavy metals, Arsenic
Aluminum Monostearate	Heavy metals
Aluminum Potassium Sulfate Hydrate	Heavy metals, Arsenic
Natural Aluminum Silicate	Heavy metals, Arsenic
Synthetic Aluminum Silicate	Heavy metals, Arsenic
Amantadine Hydrochloride	Heavy metals, Arsenic
Ambenonium Chloride	Heavy metals
Amidotrizoic Acid	Heavy metals, Arsenic
Amikacin Sulfate	Heavy metals

Official Monographs	Purity Tests to be removed
Aminophylline Hydrate	Heavy metals
Amiodarone Hydrochloride	Heavy metals
Amitriptyline Hydrochloride	Heavy metals
Amlexanox	Heavy metals
Amlodipine Besilate	Heavy metals
Ammonia Water	Heavy metals
Amobarbital	Heavy metals
Amosulalol Hydrochloride	Heavy metals
Amoxapine	Heavy metals
Amoxicillin Hydrate	Heavy metals, Arsenic
Anhydrous Ampicillin	Heavy metals, Arsenic
Ampicillin Hydrate	Heavy metals, Arsenic
Ampicillin Sodium	Heavy metals, Arsenic
Ampiroxicam	Heavy metals
Antipyrine	Heavy metals
Aprindine Hydrochloride	Heavy metals
Arbekacin Sulfate	Heavy metals
Argatroban Hydrate	Heavy metals, Arsenic
L-Arginine	Heavy metals
L-Arginine Hydrochloride	Heavy metals, Arsenic
Arotinolol Hydrochloride	Heavy metals
Ascorbic Acid	Heavy metals
L-Aspartic Acid	Heavy metals
Aspirin	Heavy metals
Aspoxicillin Hydrate	Heavy metals, Arsenic
Atenolol	Heavy metals
Atorvastatin Calcium Hydrate	Heavy metals
Auranofin	Heavy metals, Arsenic
Azathioprine	Heavy metals, Arsenic
Azelastine Hydrochloride	Heavy metals, Arsenic
Azelnidipine	Heavy metals
Azithromycin Hydrate	Heavy metals
Azosemide	Heavy metals
Aztreonam	Heavy metals
Bacampicillin Hydrochloride	Heavy metals, Arsenic
Bacitracin	Heavy metals
Baclofen	Heavy metals, Arsenic

Official Monographs	Purity Tests to be removed
Bamethan Sulfate	Heavy metals, Arsenic
Barbital	Heavy metals
Barium Sulfate	Heavy metals, Arsenic
Beclometasone Dipropionate	Heavy metals
Bekanamycin Sulfate	Heavy metals, Arsenic
Benidipine Hydrochloride	Heavy metals
Benserazide Hydrochloride	Heavy metals
Benzbromarone	Heavy metals
Benzoic Acid	Heavy metals
Benzylpenicillin Benzathine Hydrate	Heavy metals, Arsenic
Benzylpenicillin Potassium	Heavy metals, Arsenic
Bepotastine Besilate	Heavy metals
Berberine Chloride Hydrate	Heavy metals
Betahistine Mesilate	Heavy metals
Betamethasone	Heavy metals
Betamethasone Dipropionate	Heavy metals
Betamipron	Heavy metals
Betaxolol Hydrochloride	Heavy metals, Arsenic
Bethanechol Chloride	Heavy metals
Bezafibrate	Heavy metals
Bicalutamide	Heavy metals
Bifonazole	Heavy metals
Biotin	Heavy metals, Arsenic
Biperiden Hydrochloride	Heavy metals, Arsenic
Bisacodyl	Heavy metals
Bismuth Subgallate	Arsenic, Copper, Lead, Silver
Bisoprolol Fumarate	Heavy metals
Bleomycin Hydrochloride	Copper
Bleomycin Sulfate	Copper
Boric Acid	Heavy metals, Arsenic
Bromazepam	Heavy metals
Bromfenac Sodium Hydrate	Heavy metals
Bromhexine Hydrochloride	Heavy metals
Bromocriptine Mesilate	Heavy metals
Bromovalerylurea	Heavy metals, Arsenic
Brotizolam	Heavy metals
Bucillamine	Heavy metals, Arsenic

Official Monographs	Purity Tests to be removed
Bucumolol Hydrochloride	Heavy metals, Arsenic
Bufetolol Hydrochloride	Heavy metals
Buformin Hydrochloride	Heavy metals, Arsenic
Bumetanide	Heavy metals, Arsenic
Bunazosin Hydrochloride	Heavy metals
Bupivacaine Hydrochloride Hydrate	Heavy metals
Bupranolol Hydrochloride	Heavy metals, Arsenic
Buprenorphine Hydrochloride	Heavy metals
Busulfan	Heavy metals
Butenafine Hydrochloride	Heavy metals
Butropium Bromide	Heavy metals
Butyl Parahydroxybenzoate	Heavy metals
Cabergoline	Heavy metals
Cadralazine	Heavy metals
Anhydrous Caffeine	Heavy metals
Caffeine Hydrate	Heavy metals
Caffeine and Sodium Benzoate	Heavy metals, Arsenic
Precipitated Calcium Carbonate	Heavy metals, Arsenic, Barium
Calcium Chloride Hydrate	Heavy metals, Arsenic, Barium
Calcium Folinate Hydrate	Heavy metals
Calcium Gluconate Hydrate	Heavy metals, Arsenic
Calcium Hydroxide	Heavy metals, Arsenic
Calcium Lactate Hydrate	Heavy metals, Arsenic
Calcium Levofolinate Hydrate	Heavy metals, Platinum
Calcium Pantothenate	Heavy metals
Calcium Paraaminosalicylate Hydrate	Heavy metals, Arsenic
Anhydrous Dibasic Calcium Phosphate	Heavy metals
Dibasic Calcium Phosphate Hydrate	Heavy metals
Monobasic Calcium Phosphate Hydrate	Heavy metals
Calcium Polystyrene Sulfonate	Heavy metals, Arsenic
Calcium Sodium Edetate Hydrate	Heavy metals
Calcium Stearate	Heavy metals
Camostat Mesilate	Heavy metals, Arsenic
Candesartan Cilexetil	Heavy metals
Captopril	Heavy metals, Arsenic
Carbamazepine	Heavy metals
Carbazochrome Sodium Sulfonate Hydrate	Heavy metals

Official Monographs	Purity Tests to be removed
Carbidopa Hydrate	Heavy metals
L-Carbocisteine	Heavy metals, Arsenic
Carmellose	Heavy metals
Carmellose Calcium	Heavy metals
Carmellose Sodium	Heavy metals, Arsenic
Croscarmellose Sodium	Heavy metals
Carmofur	Heavy metals
Carteolol Hydrochloride	Heavy metals, Arsenic
Carumonam Sodium	Heavy metals, Arsenic
Carvedilol	Heavy metals
Cefaclor	Heavy metals, Arsenic
Cefadroxil	Heavy metals
Cefalexin	Heavy metals, Arsenic
Cefalotin Sodium	Heavy metals, Arsenic
Cefatrizine Propylene Glycolate	Heavy metals, Arsenic
Cefazolin Sodium	Heavy metals, Arsenic
Cefazolin Sodium Hydrate	Heavy metals
Cefbuperazone Sodium	Heavy metals, Arsenic
Cefcapene Pivoxil Hydrochloride Hydrate	Heavy metals
Cefdinir	Heavy metals
Cefditoren Pivoxil	Heavy metals
Cefepime Dihydrochloride Hydrate	Heavy metals
Cefmenoxime Hydrochloride	Heavy metals, Arsenic
Cefmetazole Sodium	Heavy metals, Arsenic
Cefminox Sodium Hydrate	Heavy metals, Arsenic
Cefodizime Sodium	Heavy metals, Arsenic
Cefoperazone Sodium	Heavy metals, Arsenic
Cefotaxime Sodium	Heavy metals, Arsenic
Cefotetan	Heavy metals
Cefotiam Hexetil Hydrochloride	Heavy metals, Arsenic
Cefotiam Hydrochloride	Heavy metals, Arsenic
Cefozopran Hydrochloride	Heavy metals, Arsenic
Cefpiramide Sodium	Heavy metals
Cefpirome Sulfate	Heavy metals, Arsenic
Cefpodoxime Proxetil	Heavy metals
Cefroxadine Hydrate	Heavy metals
Cefsulodin Sodium	Heavy metals, Arsenic

Official Monographs	Purity Tests to be removed
Ceftazidime Hydrate	Heavy metals
Cefteram Pivoxil	Heavy metals
Ceftibuten Hydrate	Heavy metals
Ceftizoxime Sodium	Heavy metals, Arsenic
Ceftriaxone Sodium Hydrate	Heavy metals, Arsenic
Cefuroxime Axetil	Heavy metals
Celecoxib	Heavy metals
Cellacefate	Heavy metals
Microcrystalline Cellulose	Heavy metals
Powdered Cellulose	Heavy metals
Cetirizine Hydrochloride	Heavy metals
Cetotiamine Hydrochloride Hydrate	Heavy metals
Cetraxate Hydrochloride	Heavy metals, Arsenic
Chenodeoxycholic Acid	Heavy metals, Barium
Chloramphenicol	Heavy metals
Chloramphenicol Palmitate	Heavy metals, Arsenic
Chloramphenicol Sodium Succinate	Heavy metals
Chlordiazepoxide	Heavy metals
Chlorhexidine Hydrochloride	Heavy metals, Arsenic
Chlormadinone Acetate	Heavy metals, Arsenic
Chlorphenesin Carbamate	Heavy metals, Arsenic
Chlorpheniramine Maleate	Heavy metals
d-Chlorpheniramine Maleate	Heavy metals
Chlorpromazine Hydrochloride	Heavy metals
Chlorpropamide	Heavy metals
Cibenzoline Succinate	Heavy metals, Arsenic
Ciclacillin	Heavy metals, Arsenic
Ciclosporin	Heavy metals
Cilastatin Sodium	Heavy metals, Arsenic
Cilazapril Hydrate	Heavy metals
Cilnidipine	Heavy metals
Cilostazol	Heavy metals
Cimetidine	Heavy metals, Arsenic
Cinoxacin	Heavy metals
Ciprofloxacin	Heavy metals
Ciprofloxacin Hydrochloride Hydrate	Heavy metals
Citicoline	Heavy metals, Arsenic

Official Monographs	Purity Tests to be removed
Anhydrous Citric Acid	Heavy metals
Citric Acid Hydrate	Heavy metals
Clarithromycin	Heavy metals
Clebopride Malate	Heavy metals
Clemastine Fumarate	Heavy metals, Arsenic
Clindamycin Hydrochloride	Heavy metals
Clindamycin Phosphate	Heavy metals, Arsenic
Clinofibrate	Heavy metals, Arsenic
Clobetasol Propionate	Heavy metals
Clocapramine Hydrochloride Hydrate	Heavy metals
Clofedanol Hydrochloride	Heavy metals
Clofibrate	Heavy metals, Arsenic
Clomifene Citrate	Heavy metals
Clomipramine Hydrochloride	Heavy metals, Arsenic
Clonazepam	Heavy metals
Clonidine Hydrochloride	Heavy metals, Arsenic
Cloperastine Fendizoate	Heavy metals
Cloperastine Hydrochloride	Heavy metals
Clopidogrel Sulfate	Heavy metals
Clorazepate Dipotassium	Heavy metals, Arsenic
Clotiazepam	Heavy metals, Arsenic
Clotrimazole	Heavy metals, Arsenic
Cloxacillin Sodium Hydrate	Heavy metals, Arsenic
Cloxazolam	Heavy metals, Arsenic
Colestimide	Heavy metals
Colistin Sodium Methanesulfonate	Heavy metals, Arsenic
Copovidone	Heavy metals
Croconazole Hydrochloride	Heavy metals
Crospovidone	Heavy metals
Cyanamide	Heavy metals
Cyclopentolate Hydrochloride	Heavy metals
Cyclophosphamide Hydrate	Heavy metals
Cycloserine	Heavy metals
Cyproheptadine Hydrochloride Hydrate	Heavy metals
L-Cysteine	Heavy metals
L-Cysteine Hydrochloride Hydrate	Heavy metals
L-Cystine	Heavy metals

Official Monographs	Purity Tests to be removed
Cytarabine	Heavy metals
Danazol	Heavy metals
Dantrolene Sodium Hydrate	Heavy metals
Daunorubicin Hydrochloride	Heavy metals
Deferoxamine Mesilate	Heavy metals, Arsenic
Dehydrocholic Acid	Heavy metals, Barium
Purified Dehydrocholic Acid	Heavy metals, Barium
Dehydrocholic Acid Injection	Heavy metals
Demethylchlortetracycline Hydrochloride	Heavy metals
Dexamethasone	Heavy metals
Dextran 40	Heavy metals, Arsenic
Dextran 70	Heavy metals, Arsenic
Dextran Sulfate Sodium Sulfur 5	Heavy metals, Arsenic
Dextran Sulfate Sodium Sulfur 18	Heavy metals, Arsenic
Dextrin	Heavy metals
Dextromethorphan Hydrobromide Hydrate	Heavy metals
Diazepam	Heavy metals
Dibekacin Sulfate	Heavy metals
Dibucaine Hydrochloride	Heavy metals
Diclofenac Sodium	Heavy metals, Arsenic
Diethylcarbamazine Citrate	Heavy metals
Difenidol Hydrochloride	Heavy metals, Arsenic
Diflorasone Diacetate	Heavy metals
Diflucortolone Valerate	Heavy metals
Dihydroergotoxine Mesilate	Heavy metals
Dilazep Hydrochloride Hydrate	Heavy metals, Arsenic
Diltiazem Hydrochloride	Heavy metals, Arsenic
Dimemorfan Phosphate	Heavy metals, Arsenic
Dimercaprol	Heavy metals
Dimorpholamine	Heavy metals
Diphenhydramine	Heavy metals
Diphenhydramine Hydrochloride	Heavy metals
Diphenhydramine Tannate	Heavy metals
Dipyridamole	Heavy metals, Arsenic
Disopyramide	Heavy metals, Arsenic
Distigmine Bromide	Heavy metals
Disulfiram	Heavy metals, Arsenic

Official Monographs	Purity Tests to be removed
Dobutamine Hydrochloride	Heavy metals
Docetaxel Hydrate	Heavy metals
Domperidone	Heavy metals
Donepezil Hydrochloride	Heavy metals
Dopamine Hydrochloride	Heavy metals, Arsenic
Doripenem Hydrate	Heavy metals
Dorzolamide Hydrochloride	Heavy metals
Doxapram Hydrochloride Hydrate	Heavy metals, Arsenic
Doxazosin Mesilate	Heavy metals
Doxifluridine	Heavy metals
Doxycycline Hydrochloride Hydrate	Heavy metals
Droperidol	Heavy metals
Droxidopa	Heavy metals, Arsenic
Dydrogesterone	Heavy metals
Ebastine	Heavy metals
Ecabet Sodium Hydrate	Heavy metals
Ecothiopate Iodide	Heavy metals
Edaravone	Heavy metals
Edrophonium Chloride	Heavy metals, Arsenic
Emedastine Fumarate	Heavy metals
Emorfazone	Heavy metals, Arsenic
Enalapril Maleate	Heavy metals
Enoxacin Hydrate	Heavy metals, Arsenic
Entacapone	Heavy metals
Enviomycin Sulfate	Heavy metals, Arsenic
Epalrestat	Heavy metals
Eperisone Hydrochloride	Heavy metals
Ephedrine Hydrochloride	Heavy metals
Epirizole	Heavy metals, Arsenic
Epirubicin Hydrochloride	Heavy metals
Eplerenone	Heavy metals
Eribulin Mesilate	Heavy metals
Erythromycin	Heavy metals
Estazolam	Heavy metals, Arsenic
Estriol	Heavy metals
Etacrynic Acid	Heavy metals, Arsenic
Ethambutol Hydrochloride	Heavy metals, Arsenic

Official Monographs	Purity Tests to be removed
Ethenzamide	Heavy metals, Arsenic
Ethionamide	Heavy metals, Arsenic
Ethosuximide	Heavy metals, Arsenic
Ethyl Aminobenzoate	Heavy metals
Ethylcellulose	Heavy metals
Ethyl L-Cysteine Hydrochloride	Heavy metals
Ethylenediamine	Heavy metals
Ethyl Icosapentate	Heavy metals, Arsenic
Ethyl Loflazepate	Heavy metals, Arsenic
Ethyl Parahydroxybenzoate	Heavy metals
Etidronate Disodium	Heavy metals, Arsenic
Etilefrine Hydrochloride	Heavy metals
Etizolam	Heavy metals
Etodolac	Heavy metals
Etoposide	Heavy metals
Famotidine	Heavy metals
Faropenem Sodium Hydrate	Heavy metals
Felbinac	Heavy metals
Felodipine	Heavy metals
Fenbufen	Heavy metals, Arsenic
Fenofibrate	Heavy metals
Fentanyl Citrate	Heavy metals
Ferrous Sulfate Hydrate	Heavy metals, Arsenic
Fexofenadine Hydrochloride	Heavy metals
Flavin Adenine Dinucleotide Sodium	Heavy metals, Arsenic
Flavoxate Hydrochloride	Heavy metals, Arsenic
Flecainide Acetate	Heavy metals
Flomoxef Sodium	Heavy metals, Arsenic
Flopropione	Heavy metals
Fluconazole	Heavy metals
Flucytosine	Heavy metals, Arsenic
Fludiazepam	Heavy metals
Fludrocortisone Acetate	Heavy metals
Flunitrazepam	Heavy metals
Fluorometholone	Heavy metals
Fluorouracil	Heavy metals, Arsenic
Fluphenazine Enanthate	Heavy metals

Official Monographs	Purity Tests to be removed
Flurazepam Hydrochloride	Heavy metals
Flurbiprofen	Heavy metals
Flutamide	Heavy metals
Flutoprazepam	Heavy metals
Fluvoxamine Maleate	Heavy metals
Formoterol Fumarate Hydrate	Heavy metals
Fosfomycin Calcium Hydrate	Heavy metals, Arsenic
Fosfomycin Sodium	Heavy metals, Arsenic
Fradiomycin Sulfate	Heavy metals, Arsenic
Fructose	Heavy metals, Arsenic
Fructose Injection	Heavy metals, Arsenic
Fudosteine	Heavy metals, Arsenic
Furosemide	Heavy metals
Fursultiamine Hydrochloride	Heavy metals
Gabexate Mesilate	Heavy metals, Arsenic
β-Galactosidase (Aspergillus)	Heavy metals, Arsenic
β-Galactosidase (Penicillium)	Heavy metals, Arsenic
Gatifloxacin Hydrate	Heavy metals
Gefarnate	Heavy metals
Gefitinib	Heavy metals
Gelatin	Heavy metals, Arsenic
Purified Gelatin	Heavy metals, Arsenic
Gentamicin Sulfate	Heavy metals
Glibenclamide	Heavy metals
Gliclazide	Heavy metals
Glimepiride	Heavy metals
Glucose	Heavy metals
Glucose Hydrate	Heavy metals
Purified Glucose	Heavy metals
L-Glutamic Acid	Heavy metals
L-Glutamine	Heavy metals
Glutathione	Heavy metals, Arsenic
Glycerin	Heavy metals
Concentrated Glycerin	Heavy metals
Glycine	Heavy metals, Arsenic
Guaifenesin	Heavy metals, Arsenic
Guanabenz Acetate	Heavy metals

Official Monographs	Purity Tests to be removed
Guanethidine Sulfate	Heavy metals
Haloperidol	Heavy metals
Haloxazolam	Heavy metals, Arsenic
Heparin Calcium	Heavy metals, Barium
Heparin Sodium	Barium
Heparin Sodium Injection	Barium
L-Histidine	Heavy metals
L-Histidine Hydrochloride Hydrate	Heavy metals
Homochlorcyclizine Hydrochloride	Heavy metals
Hydralazine Hydrochloride	Heavy metals
Hydrochloric Acid	Heavy metals, Arsenic, Mercury
Dilute Hydrochloric Acid	Heavy metals, Arsenic, Mercury
Hydrochlorothiazide	Heavy metals
Hydrocortisone Butyrate	Heavy metals
Hydrocortisone Sodium Phosphate	Heavy metals, Arsenic
Hydrocotarnine Hydrochloride Hydrate	Heavy metals
Hydrogenated Oil	Heavy metals
Hydroxyethylcellulose	Heavy metals
Hydroxypropylcellulose	Heavy metals
Low Substituted Hydroxypropylcellulose	Heavy metals
Hydroxyzine Hydrochloride	Heavy metals
Hydroxyzine Pamoate	Heavy metals, Arsenic
Hymecromone	Heavy metals, Arsenic
Hypromellose	Heavy metals
Hypromellose Acetate Succinate	Heavy metals
Hypromellose Phthalate	Heavy metals
Ibudilast	Heavy metals
Ibuprofen	Heavy metals, Arsenic
Ibuprofen Piconol	Heavy metals
Idarubicin Hydrochloride	Silver
Idoxuridine	Heavy metals
Ifenprodil Tartrate	Heavy metals
Imidapril Hydrochloride	Heavy metals
Imipenem Hydrate	Heavy metals, Arsenic
Indapamide	Heavy metals
Indenolol Hydrochloride	Heavy metals, Arsenic
Indigocarmine	Arsenic

Official Monographs	Purity Tests to be removed
Indometacin	Heavy metals, Arsenic
Iohexol	Heavy metals
Iopamidol	Heavy metals
Iotalamic Acid	Heavy metals, Arsenic
Iotroxic Acid	Heavy metals
Ipratropium Bromide Hydrate	Heavy metals, Arsenic
Ipriflavone	Heavy metals, Arsenic
Irbesartan	Heavy metals
Irinotecan Hydrochloride Hydrate	Heavy metals
Irsogladine Maleate	Heavy metals
Isepamicin Sulfate	Heavy metals
L-Isoleucine	Heavy metals, Arsenic
Isomalt Hydrate	Heavy metals
Isoniazid	Heavy metals, Arsenic
l-Isoprenaline Hydrochloride	Heavy metals
Isopropylantipyrine	Heavy metals, Arsenic
Isosorbide	Heavy metals, Arsenic
Isosorbide Dinitrate	Heavy metals
Isosorbide Mononitrate 70%/Lactose 30%	Heavy metals
Isoxsuprine Hydrochloride	Heavy metals
Itraconazole	Heavy metals
Josamycin	Heavy metals
Josamycin Propionate	Heavy metals
Kainic Acid Hydrate	Heavy metals, Arsenic
Kanamycin Monosulfate	Heavy metals, Arsenic
Kanamycin Sulfate	Heavy metals, Arsenic
Ketamine Hydrochloride	Heavy metals, Arsenic
Ketoconazole	Heavy metals
Ketoprofen	Heavy metals
Ketotifen Fumarate	Heavy metals
Kitasamycin Tartrate	Heavy metals
Labetalol Hydrochloride	Heavy metals
Lactic Acid	Heavy metals
L-Lactic Acid	Heavy metals
Anhydrous Lactose	Heavy metals
Lactose Hydrate	Heavy metals
Lactulose	Heavy metals, Arsenic

Official Monographs	Purity Tests to be removed
Lafutidine	Heavy metals
Lanoconazole	Heavy metals
Lansoprazole	Heavy metals, Arsenic
Latamoxef Sodium	Heavy metals, Arsenic
Lenampicillin Hydrochloride	Heavy metals, Arsenic
L-Leucine	Heavy metals, Arsenic
Levallorphan Tartrate	Heavy metals
Levodopa	Heavy metals, Arsenic
Levofloxacin Hydrate	Heavy metals
Levomepromazine Maleate	Heavy metals
Lidocaine	Heavy metals
Lincomycin Hydrochloride Hydrate	Heavy metals
Lisinopril Hydrate	Heavy metals
Lithium Carbonate	Heavy metals, Arsenic, Barium
Lobenzarit Sodium	Heavy metals, Arsenic
Lorazepam	Heavy metals, Arsenic
Losartan Potassium	Heavy metals
Loxoprofen Sodium Hydrate	Heavy metals
L-Lysine Acetate	Heavy metals
L-Lysine Hydrochloride	Heavy metals, Arsenic
Lysozyme Hydrochloride	Heavy metals
Magnesium Aluminosilicate	Heavy metals
Magnesium Aluminometasilicate	Heavy metals
Magnesium Carbonate	Heavy metals, Arsenic
Magnesium Oxide	Heavy metals
Magnesium Stearate	Heavy metals
Magnesium Sulfate Hydrate	Heavy metals, Arsenic
Maltose Hydrate	Heavy metals, Arsenic
Manidipine Hydrochloride	Heavy metals, Arsenic
D-Mannitol	Heavy metals
Maprotiline Hydrochloride	Heavy metals
Meclofenoxate Hydrochloride	Heavy metals, Arsenic
Medazepam	Heavy metals, Arsenic
Medicinal Carbon	Heavy metals, Arsenic
Medicinal Soap	Heavy metals
Medroxyprogesterone Acetate	Heavy metals
Mefenamic Acid	Heavy metals, Arsenic

Official Monographs	Purity Tests to be removed
Mefloquine Hydrochloride	Heavy metals, Arsenic
Mefruside	Heavy metals, Arsenic
Meglumine	Heavy metals
Melphalan	Heavy metals, Arsenic
Menatetrenone	Heavy metals
Mepenzolate Bromide	Heavy metals, Arsenic
Mepitiostane	Heavy metals
Mepivacaine Hydrochloride	Heavy metals
Mequitazine	Heavy metals
Mercaptopurine Hydrate	Heavy metals
Meropenem Hydrate	Heavy metals
Mesalazine	Heavy metals
Mestranol	Heavy metals, Arsenic
Metenolone Acetate	Heavy metals
Metenolone Enanthate	Heavy metals
Metformin Hydrochloride	Heavy metals
L-Methionine	Heavy metals, Arsenic
Methoxsalen	Heavy metals, Arsenic
Methylcellulose	Heavy metals
Methyldopa Hydrate	Heavy metals, Arsenic
dl-Methylephedrine Hydrochloride	Heavy metals
Methyl Parahydroxybenzoate	Heavy metals
Methylprednisolone Succinate	Heavy metals, Arsenic
Methyl Salicylate	Heavy metals
Meticrane	Heavy metals, Arsenic
Metildigoxin	Arsenic
Metoclopramide	Heavy metals, Arsenic
Metoprolol Tartrate	Heavy metals
Metronidazole	Heavy metals
Metyrapone	Heavy metals, Arsenic
Mexiletine Hydrochloride	Heavy metals
Miconazole	Heavy metals, Arsenic
Miconazole Nitrate	Heavy metals, Arsenic
Micronomicin Sulfate	Heavy metals
Midecamycin	Heavy metals
Midecamycin Acetate	Heavy metals
Miglitol	Heavy metals

Official Monographs	Purity Tests to be removed
Migrenin	Heavy metals
Minocycline Hydrochloride	Heavy metals
Mitiglinide Calcium Hydrate	Heavy metals
Mizoribine	Heavy metals
Montelukast Sodium	Heavy metals
Mosapride Citrate Hydrate	Heavy metals
Mupirocin Calcium Hydrate	Inorganic salt
Nabumetone	Heavy metals
Nadolol	Heavy metals
Nafamostat Mesilate	Heavy metals
Naftopidil	Heavy metals
Nalidixic Acid	Heavy metals
Naphazoline Nitrate	Heavy metals
Naproxen	Heavy metals, Arsenic
Nateglinide	Heavy metals
Nicardipine Hydrochloride	Heavy metals
Nicergoline	Heavy metals
Niceritrol	Heavy metals, Arsenic
Nicomol	Heavy metals, Arsenic
Nicorandil	Heavy metals
Nicotinamide	Heavy metals
Nicotinic Acid	Heavy metals
Nifedipine	Heavy metals, Arsenic
Nilvadipine	Heavy metals
Nitrazepam	Heavy metals, Arsenic
Nitrendipine	Heavy metals
Nizatidine	Heavy metals
Norfloxacin	Heavy metals, Arsenic
Norgestrel	Heavy metals
Nortriptyline Hydrochloride	Heavy metals, Arsenic
Noscapine	Heavy metals
Nystatin	Heavy metals
Ofloxacin	Heavy metals
Olmesartan Medoxomil	Heavy metals
Olopatadine Hydrochloride	Heavy metals
Omeprazole	Heavy metals
Orciprenaline Sulfate	Heavy metals

Official Monographs	Purity Tests to be removed
Oxapium Iodide	Heavy metals
Oxaprozin	Heavy metals, Arsenic
Oxazolam	Heavy metals, Arsenic
Oxethazaine	Heavy metals
Oxprenolol Hydrochloride	Heavy metals, Arsenic
Oxybuprocaine Hydrochloride	Heavy metals
Oxydol	Heavy metals, Arsenic
Oxytetracycline Hydrochloride	Heavy metals
Ozagrel Sodium	Heavy metals
Panipenem	Heavy metals
Pantethine	Heavy metals, Arsenic
Paraffin	Heavy metals
Liquid Paraffin	Heavy metals
Light Liquid Paraffin	Heavy metals
Parnaparin Sodium	Heavy metals
Paroxetine Hydrochloride Hydrate	Heavy metals
Pazufloxacin Mesilate	Heavy metals
Pemirolast Potassium	Heavy metals
Penbutolol Sulfate	Heavy metals, Arsenic
Pentazocine	Heavy metals, Arsenic
Pentobarbital Calcium	Heavy metals
Pentoxyverine Citrate	Heavy metals, Arsenic
Peplomycin Sulfate	Copper
Perphenazine	Heavy metals
Perphenazine Maleate	Heavy metals, Arsenic
White Petrolatum	Heavy metals, Arsenic
Yellow Petrolatum	Heavy metals, Arsenic
Phenethicillin Potassium	Heavy metals, Arsenic
Phenobarbital	Heavy metals
L-Phenylalanine	Heavy metals, Arsenic
Phenylbutazone	Heavy metals, Arsenic
Phenytoin	Heavy metals
Phenytoin Sodium for Injection	Heavy metals
Phytonadione	Heavy metals
Pilsicainide Hydrochloride Hydrate	Heavy metals
Pimaricin	Heavy metals
Pimozide	Heavy metals, Arsenic

The JP Drugs are to be tested according to the provisions given in the pertinent monographs, General Notices, General Rules for Crude Drugs, General Rules for Preparations, and General Tests for their conformity to the Japanese Pharmacopoeia. (*See the General Notices 5.*)

Official Monographs	Purity Tests to be removed
Pindolol	Heavy metals, Arsenic
Pioglitazone Hydrochloride	Heavy metals
Pipemidic Acid Hydrate	Heavy metals, Arsenic
Piperacillin Hydrate	Heavy metals
Piperacillin Sodium	Heavy metals, Arsenic
Piperazine Adipate	Heavy metals
Piperazine Phosphate Hydrate	Heavy metals, Arsenic
Pirarubicin	Heavy metals
Pirenoxine	Heavy metals
Pirenzepine Hydrochloride Hydrate	Heavy metals
Piroxicam	Heavy metals
Pitavastatin Calcium Hydrate	Heavy metals
Pivmecillinam Hydrochloride	Heavy metals, Arsenic
Polaprezinc	Lead
Polymixin B Sulfate	Heavy metals
Polyoxyl 40 Stearate	Heavy metals
Polysorbate 80	Heavy metals
Potassium Bromide	Heavy metals, Arsenic, Barium
Potassium Canrenoate	Heavy metals, Arsenic
Potassium Carbonate	Heavy metals, Arsenic
Potassium Chloride	Heavy metals, Arsenic
Potassium Clavulanate	Heavy metals, Arsenic
Potassium Hydroxide	Heavy metals
Potassium Iodide	Heavy metals, Arsenic, Barium
Potassium Permanganate	Arsenic
Potassium Sulfate	Heavy metals, Arsenic
Povidone	Heavy metals
Povidone-Iodine	Heavy metals, Arsenic
Pranlukast Hydrate	Heavy metals, Arsenic
Pranoprofen	Heavy metals
Prasterone Sodium Sulfate Hydrate	Heavy metals
Pravastatin Sodium	Heavy metals
Prazepam	Heavy metals, Arsenic
Prazosin Hydrochloride	Heavy metals
Prednisolone	Selenium
Prednisolone Sodium Phosphate	Heavy metals
Primidone	Heavy metals

Official Monographs	Purity Tests to be removed
Probenecid	Heavy metals, Arsenic
Probucol	Heavy metals
Procainamide Hydrochloride	Heavy metals, Arsenic
Procaine Hydrochloride	Heavy metals
Procarbazine Hydrochloride	Heavy metals
Procaterol Hydrochloride Hydrate	Heavy metals
Prochlorperazine Maleate	Heavy metals
Proglumide	Heavy metals, Arsenic
L-Proline	Heavy metals
Promethazine Hydrochloride	Heavy metals
Propafenone Hydrochloride	Heavy metals
Propiverine Hydrochloride	Heavy metals
Propranolol Hydrochloride	Heavy metals
Propylene Glycol	Heavy metals
Propyl Parahydroxybenzoate	Heavy metals
Prothionamide	Heavy metals, Arsenic
Protirelin	Heavy metals
Protirelin Tartrate Hydrate	Heavy metals, Arsenic
Pullulan	Heavy metals
Pyrantel Pamoate	Heavy metals, Arsenic
Pyrazinamide	Heavy metals
Pyridostigmine Bromide	Heavy metals, Arsenic
Pyridoxal Phosphate Hydrate	Heavy metals, Arsenic
Pyridoxine Hydrochloride	Heavy metals
Quetiapine Fumarate	Heavy metals
Quinapril Hydrochloride	Heavy metals
Quinine Ethyl Carbonate	Heavy metals
Quinine Sulfate Hydrate	Heavy metals
Rabeprazole Sodium	Heavy metals
Ranitidine Hydrochloride	Heavy metals, Arsenic
Rebamipide	Heavy metals
Ribavirin	Heavy metals, Arsenic
Riboflavin Butyrate	Heavy metals
Ribostamycin Sulfate	Heavy metals, Arsenic
Rifampicin	Heavy metals, Arsenic
Rilmazafone Hydrochloride Hydrate	Heavy metals
Ringer's Solution	Heavy metals, Arsenic

Official Monographs	Purity Tests to be removed
Risperidone	Heavy metals
Ritodrine Hydrochloride	Heavy metals
Rosuvastatin Calcium	Heavy metals
Roxatidine Acetate Hydrochloride	Heavy metals
Roxithromycin	Heavy metals
Saccharin	Heavy metals
Saccharin Sodium Hydrate	Heavy metals
Salazosulfapyridine	Heavy metals, Arsenic
Salbutamol Sulfate	Heavy metals
Salicylic Acid	Heavy metals
Sarpogrelate Hydrochloride	Heavy metals, Arsenic
Scopolamine Butylbromide	Heavy metals
L-Serine	Heavy metals
Purified Shellac	Heavy metals
White Shellac	Heavy metals
Light Anhydrous Silicic Acid	Heavy metals
Silodosin	Heavy metals
Silver Nitrate	Copper and Lead in the Purity (2) (Change the test name from ''Bismuth, copper and lead'' to ''Bismuth'')
Simvastatin	Heavy metals
Sitagliptin Phosphate Hydrate	Heavy metals
Sivelestat Sodium Hydrate	Heavy metals
Sodium Acetate Hydrate	Heavy metals, Arsenic
Sodium Aurothiomalate	Heavy metals, Arsenic
Sodium Benzoate	Heavy metals, Arsenic
Sodium Bicarbonate	Heavy metals, Arsenic
Sodium Bisulfite	Heavy metals
Sodium Borate	Heavy metals, Arsenic
Sodium Bromide	Heavy metals, Arsenic, Barium
Dried Sodium Carbonate	Heavy metals
Sodium Carbonate Hydrate	Heavy metals
Sodium Chloride	Heavy metals
Isotonic Sodium Chloride Solution	Heavy metals, Arsenic
Sodium Citrate Hydrate	Heavy metals, Arsenic
Sodium Cromoglicate	Heavy metals
Disodium Edetate Hydrate	Heavy metals, Arsenic
Sodium Fusidate	Heavy metals

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Official Monographs	Purity Tests to be removed
Purified Sodium Hyaluronate	Heavy metals
Sodium Hydroxide	Heavy metals, Mercury
Sodium Iodide	Heavy metals
Sodium L-Lactate Solution	Heavy metals, Arsenic
Sodium L-Lactate Ringer's Solution	Heavy metals
Dibasic Sodium Phosphate Hydrate	Heavy metals
Sodium Picosulfate Hydrate	Heavy metals, Arsenic
Sodium Polystyrene Sulfonate	Heavy metals, Arsenic
Sodium Pyrosulfite	Heavy metals
Sodium Risedronate Hydrate	Heavy metals, Arsenic
Sodium Salicylate	Heavy metals, Arsenic
Sodium Starch Glycolate	Heavy metals
Dried Sodium Sulfite	Heavy metals
Sodium Thiosulfate Hydrate	Heavy metals, Arsenic
Sodium Valproate	Heavy metals
Sorbitan Sesquioleate	Heavy metals
D-Sorbitol	Heavy metals, Arsenic, Nickel
D-Sorbitol Solution	Heavy metals, Arsenic, Nickel
Spiramycin Acetate	Heavy metals
Stearic Acid	Heavy metals
Streptomycin Sulfate	Heavy metals, Arsenic
Sucralfate Hydrate	Heavy metals, Arsenic
White Soft Sugar	Heavy metals
Sulbactam Sodium	Heavy metals
Sulbenicillin Sodium	Heavy metals, Arsenic
Sulfamethizole	Heavy metals, Arsenic
Sulfamethoxazole	Heavy metals, Arsenic
Sulfamonomethoxine Hydrate	Heavy metals, Arsenic
Sulfisoxazole	Heavy metals
Sulfobromophthalein Sodium	Heavy metals, Arsenic
Sulfur	Arsenic
Sulindac	Heavy metals, Arsenic
Sulpiride	Heavy metals
Sulpyrine Hydrate	Heavy metals
Sultamicillin Tosilate Hydrate	Heavy metals
Sultiame	Heavy metals, Arsenic
Tacrolimus Hydrate	Heavy metals

Official Monographs	Purity Tests to be removed
Talampicillin Hydrochloride	Heavy metals, Arsenic
Taltirelin Hydrate	Heavy metals
Tamoxifen Citrate	Heavy metals
Tamsulosin Hydrochloride	Heavy metals
Tartaric Acid	Heavy metals, Arsenic
Taurine	Heavy metals
Tazobactam	Heavy metals
Tegafur	Heavy metals, Arsenic
Teicoplanin	Heavy metals, Arsenic
Telmisartan	Heavy metals
Temocapril Hydrochloride	Heavy metals
Teprenone	Heavy metals
Terbinafine Hydrochloride	Heavy metals
Terbutaline Sulfate	Heavy metals, Arsenic
Tetracaine Hydrochloride	Heavy metals
Tetracycline Hydrochloride	Heavy metals
Theophylline	Heavy metals, Arsenic
Thiamazole	Heavy metals, Arsenic, Selenium
Thiamine Chloride Hydrochloride	Heavy metals
Thiamine Nitrate	Heavy metals
Thiamylal Sodium	Heavy metals
Thiopental Sodium	Heavy metals
Thiopental Sodium for Injection	Heavy metals
Thioridazine Hydrochloride	Heavy metals, Arsenic
L-Threonine	Heavy metals, Arsenic
Tiapride Hydrochloride	Heavy metals
Tiaramide Hydrochloride	Heavy metals, Arsenic
Ticlopidine Hydrochloride	Heavy metals, Arsenic
Timepidium Bromide Hydrate	Heavy metals
Timolol Maleate	Heavy metals
Tinidazole	Heavy metals, Arsenic
Tipepidine Hibenzate	Heavy metals, Arsenic
Tizanidine Hydrochloride	Heavy metals
Tobramycin	Heavy metals
Tocopherol	Heavy metals
Tocopherol Acetate	Heavy metals
Tocopherol Nicotinate	Heavy metals, Arsenic

Official Monographs	Purity Tests to be removed
Todralazine Hydrochloride Hydrate	Heavy metals, Arsenic
Tofisopam	Heavy metals, Arsenic
Tolbutamide	Heavy metals
Tolnaftate	Heavy metals
Tolperisone Hydrochloride	Heavy metals
Tosufloxacin Tosilate Hydrate	Heavy metals, Arsenic
Tramadol Hydrochloride	Heavy metals
Tranexamic Acid	Heavy metals, Arsenic
Tranilast	Heavy metals
Trapidil	Heavy metals, Arsenic
Trehalose Hydrate	Heavy metals
Trepibutone	Heavy metals
Triamcinolone	Heavy metals
Triamcinolone Acetonide	Heavy metals
Triamterene	Heavy metals, Arsenic
Triazolam	Heavy metals
Trichlormethiazide	Heavy metals, Arsenic
Triclofos Sodium	Heavy metals, Arsenic
Trientine Hydrochloride	Heavy metals
Trihexyphenidyl Hydrochloride	Heavy metals
Trimebutine Maleate	Heavy metals, Arsenic
Trimetazidine Hydrochloride	Heavy metals
Trimethadione	Heavy metals
Trimetoquinol Hydrochloride Hydrate	Heavy metals
Tropicamide	Heavy metals
Troxipide	Heavy metals
L-Tryptophan	Heavy metals, Arsenic
Tulobuterol	Heavy metals
Tulobuterol Hydrochloride	Heavy metals
L-Tyrosine	Heavy metals
Ubenimex	Heavy metals
Ubidecarenone	Heavy metals
Ulinastatin	Heavy metals
Urapidil	Heavy metals
Urea	Heavy metals
Urokinase	Heavy metals
Ursodeoxycholic Acid	Heavy metals, Barium

Official Monographs	Purity Tests to be removed
Valaciclovir Hydrochloride	Heavy metals, Palladium
L-Valine	Heavy metals, Arsenic
Valsartan	Heavy metals
Vancomycin Hydrochloride	Heavy metals
Verapamil Hydrochloride	Heavy metals, Arsenic
Voglibose	Heavy metals
Voriconazole	Heavy metals
Warfarin Potassium	Heavy metals
Wine	Arsenic
Xylitol	Heavy metals, Arsenic, Nickel
Zaltoprofen	Heavy metals, Arsenic
Zidovudine	Heavy metals
Zinc Chloride	Heavy metals, Arsenic
Zinc Oxide	Lead, Arsenic
Zinc Sulfate Hydrate	Heavy metals, Arsenic
Zolpidem Tartrate	Heavy metals
Zonisamide	Heavy metals
Zopiclone	Heavy metals

Amphotericin B for Injection

注射用アムホテリシン **B**

Change the description of Uniformity of dosage units as follows:

Uniformity of dosage units ⟨*6.02*⟩ It meets the requirement of the Mass variation test (*T*: Being specified separately when the drug is granted approval based on the Law.).

Amphotericin B Tablets

アムホテリシン **B** 錠

Change the description of Uniformity of dosage units as follows:

Uniformity of dosage units ⟨*6.02*⟩ It meets the requirement of the Mass variation test (*T*: Being specified separately when the drug is granted approval based on the Law.).

Ampicillin Sodium and Sulbactam Sodium for Injection

注射用アンピシリンナトリウム·スルバクタムナトリウム

Change the description of Uniformity of dosage units as follows:

Uniformity of dosage units ⟨*6.02*⟩ Perform the test according to the following method: it meets the requirement of the Content uniformity test (*T*: Being specified separately when the drug is granted approval based on the Law.).

Dissolve 1 Ampicillin Sodium and Sulbactam Sodium for Injection in the mobile phase to make exactly *V* mL so that each mL contains 5 mg (potency) of ampicillin ($C_{16}H_{19}N_3O_4S$). Pipet 5 mL of this solution, add exactly 5 mL of the internal standard solution, then add the mobile phase to make 50 mL, and use this solution as the sample solution. Then, proceed as directed in the Assay.

Amount [mg (potency)] of ampicillin ($C_{16}H_{19}N_3O_4S$)
$= M_{S1} \times Q_{Ta}/Q_{Sa} \times V/10$

Amount [mg (potency)] of sulbactam ($C_8H_{11}NO_5S$)
$= M_{S2} \times Q_{Tb}/Q_{Sb} \times V/10$

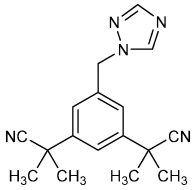
M_{S1}: Amount [mg (potency)] of Ampicillin RS taken
M_{S2}: Amount [mg (potency)] of Sulbactam RS taken

Internal standard solution—A solution of parahydroxybenzoic acid in the mobile phase (1 in 1000).

Add the following:

Anastrozole

アナストロゾール



$C_{17}H_{19}N_5$: 293.37
2,2′-[5-(1*H*-1,2,4-Triazol-1-ylmethyl)benzene-1,3-diyl]bis(2-methylpropanenitrile)
[*120511-73-1*]

Anastrozole contains not less than 98.0% and not more than 102.0% of anastrozole ($C_{17}H_{19}N_5$).

Description Anastrozole occurs as a white, crystalline powder or powder.

It is very soluble in acetonitrile, freely soluble in methanol and in ethanol (99.5), and very slightly soluble in water.

It shows crystal polymorphism.

Identification (1) Determine the absorption spectrum of a solution of Anastrozole in methanol (1 in 50,000) as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Anastrozole RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Anastrozole as directed in the potassium bromide disk method under Infrared Spectrophotometry ⟨*2.25*⟩, and compare the spectrum with the Reference Spectrum or the spectrum of Anastrozole RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

Purity Related substances—Weigh accurately about 50 mg of Anastrozole, add 10 mL of acetonitrile for liquid chromatography, sonicate to dissolve, add the mobile phase A to make exactly 25 mL, and use this solution as the sample solution. Separately, weigh accurately about 50 mg of Anastrozole RS, add 10 mL of acetonitrile for liquid chromatography, sonicate to dissolve, and add the mobile phase A to make exactly 25 mL. Pipet 1 mL of this solution, add the mobile phase A to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions. Determine the peak area, A_T, of each related substance from the sample solution, and the peak area, A_S, of anastrozole from the standard solution by the automatic integration method, and calculate the amounts of related substances by the following equation: the amounts of the related substances A and B, having the relative retention time of about 0.63 and about 2.2 to anastrozole, obtained from the sample solution are not more than 0.2%, respectively, each of other related substances is not more than 0.1%, and the total amount of other related substances is not more than 0.2%. Furthermore, the total amount of the related substances is not more than 0.5%.

$$\text{Amount (\%) of related substance} = M_S/M_T \times A_T/A_S$$

M_S: Amount (mg) of Anastrozole RS taken
M_T: Amount (mg) of Anastrozole taken

Operating conditions—

Detector, column, column temperature, mobile phases A and B, flowing of mobile phase and flow rate: Proceed as directed in the operating conditions in the Assay.

Time span of measurement: For 40 minutes after injection of the sample solution.

System suitability—

Test for required detectability: Pipet 1 mL of the standard solution, and add the mobile phase A to make exactly 20 mL. Confirm that the peak area of anastrozole obtained with 10 μL of this solution is equivalent to 3 to 7% of that with 10 μL of the standard solution.

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of anastrozole are not less than 1500 and not more than 1.4, respectively.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of anastrozole is not more than 2.0%.

Water ⟨*2.48*⟩ Not more than 0.3% (50 mg, coulometric titration).

Residue on ignition ⟨*2.44*⟩ Not more than 0.1% (1 g).

Assay Weigh accurately about 25 mg each of Anastrozole and Anastrozole RS, to each add 20 mL of acetonitrile for liquid chromatography, sonicate to dissolve, add the mobile phase A to make exactly 50 mL, and use these solutions as the sample solution and the standard solution, respectively. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, and determine the peak areas, A_T and A_S, of anastrozole in each solution.

$$\text{Amount (mg) of anastrozole } (C_{17}H_{19}N_5) = M_S \times A_T/A_S$$

M_S: Amount (mg) of Anastrozole RS taken

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 215 nm).

Column: A stainless steel column 3.2 mm in inside diameter and 10 cm in length, packed with octadecylsilyl and octylsilyl groups bound to porous silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase A: A mixture of water, methanol for liquid chromatography, acetonitrile for liquid chromatography and trifluoroacetic acid (1200:600:200:1).

Mobile phase B: A mixture of methanol for liquid chromatography, water, acetonitrile for liquid chromatography and trifluoroacetic acid (900:800:300:1).

Flowing of mobile phase: Control the gradient by mixing the mobile phases A and B as directed in the following table.

Time after injection of sample (min)	Mobile phase A (vol%)	Mobile phase B (vol%)
0 – 10	100	0
10 – 40	100 → 0	0 → 100

Flow rate: 0.75 mL per minute (the retention time of anastrozole is about 6 minutes).

System suitability—

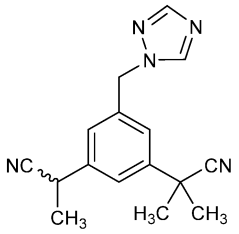
System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of anastrozole are not less than 1200 and not more than 1.4, respectively.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of anastrozole is not more than 1.0%.

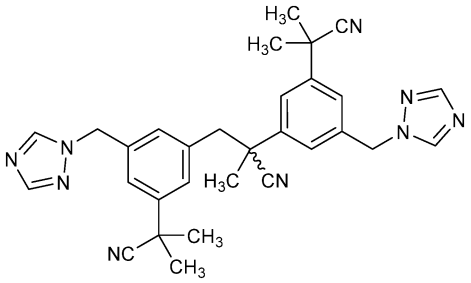
Containers and storage Containers—Well-closed containers.

Others

Related substance A:
2-[3-(1-Cyanoethyl)-5-(1*H*-1,2,4-triazol-1-ylmethyl)phenyl]-2-methylpropanenitrile



Related substance B:
2,3-Bis[3-(2-cyanopropan-2-yl)-5-(1*H*-1,2,4-triazol-1-ylmethyl)phenyl]-2-methylpropanenitrile



Add the following:

Anastrozole Tablets

アナストロゾール錠

Anastrozole Tablets contain not less than 95.0% and not more than 105.0% of the labeled amount of anastrozole ($C_{17}H_{19}N_5$: 293.37).

Method of preparation Prepare as directed under Tablets, with Anastrozole.

Identification To a quantity of powdered Anastrozole Tablets, equivalent to 8 mg of Anastrozole, add 10 mL of diethyl ether, sonicate, and filter through a membrane filter with a pore size not exceeding 0.45 μm. To the filtrate add 0.40 g of potassium bromide for infrared spectrophotometry, and evaporate the diethyl ether. Determine the infrared absorption spectrum of the residue as directed in the potassium bromide disk method under Infrared Spectrophotometry <*2.25*>: it exhibits absorption at the wave numbers of about 3100 cm^{-1}, 2980 cm^{-1}, 2240 cm^{-1}, 1606 cm^{-1}, 1502 cm^{-1}, 1359 cm^{-1}, 1206 cm^{-1}, 1139 cm^{-1}, 876 cm^{-1}, 763 cm^{-1}, 713 cm^{-1} and 680 cm^{-1}.

Uniformity of dosage units <*6.02*> Perform the test according to the following method: it meets the requirement of the Content uniformity test.

To 1 tablet of Anastrozole Tablets add 8 mL of a mixture of water, acetonitrile for liquid chromatography and trifluoroacetic acid (1000:1000:1), sonicate, and shake thoroughly until the tablet is completely disintegrated. Add a mixture of water, acetonitrile for liquid chromatography and trifluoroacetic acid (1000:1000:1) to make exactly V mL so that each mL contains about 0.1 mg of anastrozole ($C_{17}H_{19}N_5$). Filter this solution through a membrane filter with a pore size not exceeding 0.45 μm, discard 3 mL of the first filtrate, and use the subsequent filtrate as the sample solution. Then, proceed as directed in the Assay.

$$\text{Amount (mg) of anastrozole } (C_{17}H_{19}N_5) = M_S \times A_T/A_S \times V/500$$

M_S: Amount (mg) of Anastrozole RS taken

Dissolution ⟨*6.10*⟩ When the test is performed at 50 revolutions per minute according to the Paddle method, using 1000 mL of water as the dissolution medium, the dissolution rate in 15 minutes of Anastrozole Tablets is not less than 80%.

Start the test with 1 tablet of Anastrozole Tablets, withdraw not less than 10 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard not less than 3 mL of the first filtrate, pipet V mL of the subsequent filtrate, add water to make exactly V' mL so that each mL contains about 1.0 μg of anastrozole ($C_{17}H_{19}N_5$), and use this solution as the sample solution. Separately, weigh accurately about 50 mg of Anastrozole RS, add 20 mL of acetonitrile for liquid chromatography, sonicate, and add water to make exactly 250 mL. Pipet 5 mL of this solution, add water to make exactly 100 mL. Pipet 10 mL of this solution, add water to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 100 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, and determine the peak areas, A_T and A_S, of anastrozole in each solution.

Dissolution rate (%) with respect to the labeled amount of anastrozole ($C_{17}H_{19}N_5$)
$= M_S \times A_T/A_S \times V'/V \times 1/C \times 2$

M_S: Amount (mg) of Anastrozole RS taken
C: Labeled amount (mg) of anastrozole ($C_{17}H_{19}N_5$) in 1 tablet

Operating conditions—

Detector, column and column temperature: Proceed as directed in the operating conditions in the Assay under Anastrozole.

Mobile phase: A mixture of water, acetonitrile for liquid chromatography and trifluoroacetic acid (700:300:1).

Flow rate: Adjust so that the retention time of anastrozole is about 7 minutes.

System suitability—

System performance: To 15 mg of methyl parahydroxybenzoate and 50 mg of Anastrozole RS, add 20 mL of acetonitrile for liquid chromatography, sonicate, and add water to make 250 mL. To 5 mL of this solution add water to make 100 mL. To 10 mL of this solution add water to make 100 mL, and use this solution as the solution for system suitability test. When the procedure is run with 100 μL of the solution for system suitability test under the above operating conditions, methyl parahydroxybenzoate and anastrozole are eluted in this order with the resolution between these peaks being not less than 4.

System repeatability: When the test is repeated 6 times with 100 μL of the solution for system suitability test under the above operating conditions, the relative standard deviation of the peak area of anastrozole is not more than 1.5%.

Assay Weigh accurately the mass of not less than 20 tablets of Anastrozole Tablets, and powder. Weigh accurately a portion of the powder, equivalent to about 10 mg of anastrozole ($C_{17}H_{19}N_5$), add 80 mL of a mixture of water, acetonitrile for liquid chromatography and trifluoroacetic acid (1000:1000:1), sonicate to dissolve, and add a mixture of water, acetonitrile for liquid chromatography and trifluoroacetic acid (1000:1000:1) to make exactly 100 mL. Filter this solution through a membrane filter with a pore size not exceeding 0.45 μm, discard 3 mL of the first filtrate, and use the subsequent filtrate as the sample solution. Separately, weigh accurately about 50 mg of Anastrozole RS, add 50 mL of a mixture of water, acetonitrile for liquid chromatography and trifluoroacetic acid (1000:1000:1), sonicate to dissolve, and add a mixture of water, acetonitrile for liquid chromatography and trifluoroacetic acid (1000:1000:1) to make exactly 100 mL. Pipet 10 mL of this solution, add a mixture of water, acetonitrile for liquid chromatography and trifluoroacetic acid (1000:1000:1) to make exactly 50 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, and determine the peak areas, A_T and A_S, of anastrozole in each solution.

Amount (mg) of anastrozole ($C_{17}H_{19}N_5$)
$= M_S \times A_T/A_S \times 1/5$

MS: Amount (mg) of Anastrozole RS taken

Operating conditions—

Detector, column and column temperature: Proceed as directed in the operating conditions in the Assay under Anastrozole.

Mobile phase: A mixture of water, methanol for liquid chromatography, acetonitrile for liquid chromatography and trifluoroacetic acid (7000:2000:1000:7).

Flow rate: Adjust so that the retention time of anastrozole is about 15 minutes.

System suitability—

System performance: To 30 mg of ethyl parahydroxybenzoate and 50 mg of Anastrozole RS, add 50 mL of a mixture of water, acetonitrile for liquid chromatography and trifluoroacetic acid (1000:1000:1), sonicate to dissolve, and add a mixture of water, acetonitrile for liquid chromatography and trifluoroacetic acid (1000:1000:1) to make 100 mL. To 10 mL of this solution add a mixture of water, acetonitrile for liquid chromatography and trifluoroacetic acid (1000:1000:1) to make 50 mL, and use this solution as the solution for system suitability test. When the procedure is run with 10 μL of the solution for system suitability test under the above operating conditions, ethyl parahydroxybenzoate and anastrozole are eluted in this order with the resolution between these peaks being not less than 4.

System repeatability: When the test is repeated 6 times with 10 μL of the solution for system suitability test under the above operating conditions, the relative standard deviation of the peak area of anastrozole is not more than 1.5%.

Containers and storage Containers—Tight containers.

Benzyl Alcohol

ベンジルアルコール

Change the Identification as follows:

Identification Determine the infrared absorption spectrum of Benzyl Alcohol as directed in the liquid film method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Add the following:

Bicalutamide Tablets

ビカルタミド錠

Bicalutamide Tablets contain not less than 95.0% and not more than 105.0% of the labeled amount of bicalutamide ($C_{18}H_{14}F_4N_2O_4S$: 430.37).

Method of preparation Prepare as directed under Tablets, with Bicalutamide.

Identification To a quantity of powdered Bicalutamide Tablets, equivalent to 5 mg of Bicalutamide, add 250 mL of methanol, shake thoroughly, and filter through a membrane filter with a pore size not exceeding 0.45 μm. To 10 mL of the filtrate add methanol to make 20 mL. Determine the absorption spectrum of the solution as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits a maximum between 269 nm and 273 nm.

Uniformity of dosage units <6.02> Perform the Mass variation test, or the Content uniformity test according to the following method: it meets the requirement.

To 1 tablet of Bicalutamide Tablets add 10 mL of water, shake until the tablet is disintegrated. Then, add 80 mL of tetrahydrofuran, sonicate, add tetrahydrofuran to make exactly 100 mL, and filter through a membrane filter with a pore size 0.45 μm. Discard 1 mL of the first filtrate, pipet V mL of the subsequent filtrate, add a solution of sodium lauryl sulfate (3 in 200) to make exactly V' mL so that each mL contains about 8 μg of bicalutamide ($C_{18}H_{14}F_4N_2O_4S$), and use this solution as the sample solution. Separately, weigh accurately about 16 mg of Bicalutamide RS (separately determine the loss on drying <2.41> in the same conditions as Bicalutamide), dissolve in 2 mL of tetrahydrofuran, and add a solution of sodium lauryl sulfate (3 in 200) to make exactly 200 mL. Pipet 5 mL of this solution, add a solution of sodium lauryl sulfate (3 in 200) to make exactly 50 mL, and use this solution as the standard solution. Determine the absorbances, A_T and A_S, of the sample solution and standard solution at 270 nm as directed under Ultraviolet-visible Spectrophotometry <2.24>.

Amount (mg) of bicalutamide ($C_{18}H_{14}F_4N_2O_4S$)
$= M_S \times A_T/A_S \times V'/V \times 1/20$

M_S: Amount (mg) of Bicalutamide RS taken, calculated on the dried basis

Dissolution <6.10> When the test is performed at 50 revolutions per minute according to the Paddle method, using 1000 mL of a solution of sodium lauryl sulfate (3 in 200) as the dissolution medium, the dissolution rate in 45 minutes of Bicalutamide Tablets is not less than 80%.

Start the test with 1 tablet of Bicalutamide Tablets, withdraw not less than 10 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard not less than 1 mL of the first filtrate, pipet V mL of the subsequent filtrate, add the dissolution medium to make exactly V' mL so that each mL contains about 8 μg of bicalutamide ($C_{18}H_{14}F_4N_2O_4S$), and use this solution as the sample solution. Separately, weigh accurately about 16 mg of Bicalutamide RS (separately determine the loss on drying <2.41> in the same conditions as Bicalutamide), dissolve in 2 mL of tetrahydrofuran, and add the dissolution medium to make exactly 200 mL. Pipet 5 mL of this solution, add the dissolution medium to make exactly 50 mL, and use this solution as the standard solution. Determine the absorbances, A_T and A_S, of the sample solution and standard solution at 270 nm as directed under Ultraviolet-visible Spectrophotometry <2.24>.

Dissolution rate (%) with respect to the labeled amount of bicalutamide ($C_{18}H_{14}F_4N_2O_4S$)
$= M_S \times A_T/A_S \times V'/V \times 1/C \times 50$

M_S: Amount (mg) of Bicalutamide RS taken, calculated on the dried basis

C: Labeled amount (mg) of bicalutamide ($C_{18}H_{14}F_4N_2O_4S$) in 1 tablet

Assay Weigh accurately the mass of not less than 20 tablets of Bicalutamide Tablets, and powder. Weigh accurately a portion of the powder, equivalent to about 50 mg of bicalutamide ($C_{18}H_{14}F_4N_2O_4S$), add 50 mL of tetrahydrofuran, sonicate, and add tetrahydrofuran to make exactly 100 mL. Filter this solution through a membrane filter with a pore size not exceeding 0.45 μm. Discard 1 mL of the first filtrate, pipet 4 mL of the subsequent filtrate, add exactly 5 mL of the internal standard solution, then add the mobile phase to make 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 25 mg of Bicalutamide RS (separately determine the loss on drying <2.41> in the same conditions as Bicalutamide), dissolve in tetrahydrofuran to make exactly 50 mL. Pipet 4 mL of this solution, add exactly 5 mL of the internal standard solution, then add the mobile phase to make 50 mL, and use this solution as the standard solution. Perform the test with 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of bicalutamide to that of the internal

standard.

$$\text{Amount (mg) of bicalutamide } (C_{18}H_{14}F_4N_2O_4S) = M_S \times Q_T/Q_S \times 2$$

M_S: Amount (mg) of Bicalutamide RS taken, calculated on the dried basis

Internal standard solution—A solution of propyl parahydroxybenzoate in the mobile phase.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 270 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 12.5 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (3 μm in particle diameter).

Column temperature: A constant temperature of about 50°C.

Mobile phase: A mixture of water, tetrahydrofuran and acetonitrile (13:4:3).

Flow rate: Adjust so that the retention time of bicalutamide is about 7 minutes.

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the internal standard and bicalutamide are eluted in this order with the resolution between these peaks being not less than 7.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of bicalutamide to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Well-closed containers.

Bromhexine Hydrochloride

ブロムヘキシン塩酸塩

Change the Purity as follows:

Purity Related substances—Conduct this procedure without exposure to light, using light-resistant vessels. Dissolve 50 mg of Bromhexine Hydrochloride in 10 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of the sample solution, and add the mobile phase to make exactly 20 mL. Pipet 1 mL of this solution, add the mobile phase to make exactly 25 mL, and use this solution as the standard solution. Perform the test with exactly 5 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine each peak area by the automatic integration method: each peak area other than bromhexine obtained from the sample solution is not larger than the peak area of bromhexine from the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 245 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: Dissolve 1.0 g of potassium dihydrogen phosphate in 900 mL of water, adjust to pH 7.0 with 0.5 mol/L sodium hydroxide TS, and add water to make 1000 mL. To 200 mL of this solution add 800 mL of acetonitrile.

Flow rate: Adjust so that the retention time of bromhexine is about 6 minutes.

Time span of measurement: About 2 times as long as the retention time of bromhexine, beginning after the solvent peak.

System suitability—

Test for required detectability: Pipet 5 mL of the standard solution, and add the mobile phase to make exactly 20 mL. Confirm that the peak area of bromhexine obtained with 5 μL of this solution is equivalent to 17.5 to 32.5% of that with 5 μL of the standard solution.

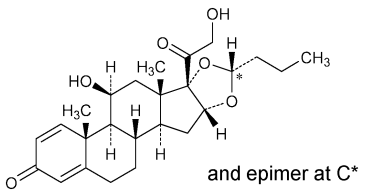
System performance: When the procedure is run with 5 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of bromhexine are not less than 2800 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 5 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of bromhexine is not more than 2.0%.

Add the following:

Budesonide

ブデソニド



$C_{25}H_{34}O_6$: 430.53

16α,17-[(1*RS*)-Butylidenebis(oxy)]-11β,21-dihydroxypregna-1,4-diene-3,20-dione

[*51333-22-3*]

Budesonide contains not less than 98.0% and not more than 102.0% of budesonide ($C_{25}H_{34}O_6$), calculated on the dried basis.

Description Budesonide occurs as white to pale yellow-white, crystals or crystalline powder.

It is soluble in methanol, sparingly soluble in acetonitrile and in ethanol (99.5), and practically insoluble in water.

Optical rotation: $[\alpha]_D^{25}$: +102 – +109° (0.25 g, methanol, 25 mL, 100 mm).

Melting point: about 240°C (with decomposition).

Identification **(1)** Determine the absorption spectrum of a solution of Budesonide (1 in 40,000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Budesonide RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Budesonide as directed in the potassium bromide disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum or the spectrum of Budesonide RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

Purity Related substances—Conduct this procedure without exposure to light, using light-resistant vessels. Dissolve 50 mg of Budesonide in 15 mL of acetonitrile, add phosphate buffer solution (pH 3.2) to make 50 mL, and use this solution as the sample solution. Perform the test with 20 μL of the sample solution as directed under Liquid Chromatography <*2.01*> according to the following conditions. Determine each peak area by the automatic integration method, and calculate the amount of them by the area percentage method: the amounts of the peaks of related substances A and L, having the relative retention times of about 0.1 and about 0.95 to the first eluted peak (epimer B) of the two peaks of budesonide, are not more than 0.2%, respectively, the sum of the amounts of the peaks of related substance D, having the relative retention times of about 0.63 and about 0.67, and the sum of the amounts of the peaks of the related substance K, having the relative retention times of about 2.9 and about 3.0, are not more than 0.2%, respectively, and the amount of the peak other than budesonide and mentioned above is not more than 0.1%. Furthermore, the total amount of the peaks other than budesonide is not more than 0.5%. For the peak areas of the related substances D and K, multiply their correction factors, 1.8 and 1.3, respectively.

Operating conditions—

Detector, column, column temperature and flow rate: Proceed as directed in the operating conditions in the Assay.

Mobile phase A: A mixture of phosphate buffer solution (pH 3.2), acetonitrile for liquid chromatography and ethanol (99.5) (34:16:1).

Mobile phase B: A mixture of phosphate buffer solution (pH 3.2) and acetonitrile for liquid chromatography (1:1).

Flowing of mobile phase: Control the gradient by mixing the mobile phases A and B as directed in the following table.

Time after injection of sample (min)	Mobile phase A (vol%)	Mobile phase B (vol%)
0 – 38	100	0
38 – 50	100 → 0	0 → 100
50 – 60	0	100

Time span of measurement: For 60 minutes after injection, beginning after the solvent peak.

System suitability—

Test for required detectability: Pipet 1 mL of the sample solution add a mixture of phosphate buffer solution (pH 3.2) and acetonitrile (17:8) to make exactly 10 mL. Pipet 1 mL of this solution, add a mixture of phosphate buffer solution (pH 3.2) and acetonitrile (17:8) to make exactly 100 mL, and use this solution as the solution for system suitability test. When the procedure is run with 20 μL of the solution for system suitability test under the above operating conditions, the SN ratio of the second eluted peak (epimer A) of the two peaks of budesonide is not less than 10.

System performance: When the procedure is run with 20 μL of the solution for system suitability test under the above operating conditions, the resolution between the two peaks of budesonide is not less than 1.5.

Loss on drying <*2.41*> Not more than 0.5% (1 g, 105°C, 3 hours).

Isomer ratio Conduct this procedure without exposure to light, using light-resistant vessels. Perform the test with 20 μL of the sample solution obtained in the Assay as directed under Liquid Chromatography <*2.01*> according to the following conditions. Determine the peak areas, A_b and A_a, where A_b is the area of the early eluted peak and A_a is the area of the lately eluted peak of the two peaks of budesonide: $A_a/(A_a + A_b)$ is between 0.40 and 0.51.

Operating conditions—

Proceed as directed in the operating conditions in the Assay.

System suitability—

System performance: Proceed as directed in the system suitability in the Assay.

Assay Conduct this procedure without exposure to light, using light-resistant vessels. Weigh accurately about 25 mg each of Budesonide and Budesonide RS (separately determine the loss on drying <*2.41*> under the same conditions as Budesonide), dissolve each in 15 mL of acetonitrile, add phosphate buffer solution (pH 3.2) to make exactly 50 mL, and use these solutions as the sample solution and the standard solution, respectively. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine the peak areas, A_T and A_S, of the sum of the two peak areas of budesonide in each solution.

Amount (mg) of budesonide ($C_{25}H_{34}O_6$) = $M_S \times A_T/A_S$

M_S: Amount (mg) of Budesonide RS taken, calculated on the dried basis

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 240 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (3 μm in particle diameter).

Column temperature: A constant temperature of about 50°C.

Mobile phase: A mixture of phosphate buffer solution (pH 3.2), acetonitrile for liquid chromatography and ethanol (99.5) (34:16:1).

Flow rate: 1.0 mL per minute (the retention times of two peaks of budesonide are about 17 and about19 minutes).

System suitability—

System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, the resolution between the two peaks of budesonide is not less than 1.5.

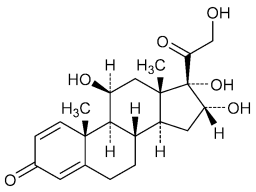
System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the sum of the two peak areas of budesonide is not more than 1.0%.

Containers and storage Containers—Tight containers.

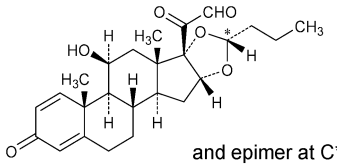
Storage—Light-resistant.

Others

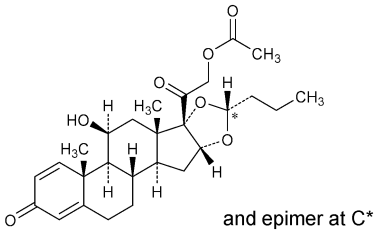
Related substance A:
11β,16α,17,21-Tetrahydroxypregna-1,4-diene-3,20-dione



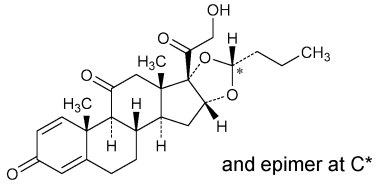
Related substance D:
16α,17-[(1*RS*)-Butylidenebis(oxy)]-11β-hydroxy-3,20-dioxopregna-1,4-dien-21-al



Related substance K:
16α,17-[(1*RS*)-Butylidenebis(oxy)]-11β,21-dihydroxypregna-1,4-diene-3,20-dione 21-acetate



Related substance L:
16α,17-[(1*RS*)-Butylidenebis(oxy)]-21-hydroxypregna-1,4-diene-3,11,20-trione



Butropium Bromide

ブトロピウム臭化物

Change the Assay as follows:

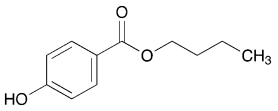
Assay Weigh accurately about 0.8 g of Butropium Bromide, previously dried, dissolve in 5 mL of formic acid, add 100 mL of acetic anhydride, and titrate <*2.50*> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 53.25 mg of $C_{28}H_{38}BrNO_4$

Change the following as follows:

Butyl Parahydroxybenzoate

パラオキシ安息香酸ブチル



$C_{11}H_{14}O_3$: 194.23
Butyl 4-hydroxybenzoate
[*94-26-8*]

This monograph is harmonized with the European Pharmacopoeia and the U.S. Pharmacopeia.

The corresponding part of the attributes/provisions which are agreed as non-harmonized within the scope of the harmonization is marked with symbols (◆ ◆), and the corresponding parts which are agreed as the JP local require-

ment other than the scope of the harmonization are marked with symbols (◇ ◇).

Information on the harmonization with the European Pharmacopoeia and the U.S. Pharmacopeia is available on the website of the Pharmaceuticals and Medical Devices Agency.

Butyl Parahydroxybenzoate contains not less than 98.0% and not more than 102.0% of butyl parahydroxybenzoate ($C_{11}H_{14}O_3$).

◆**Description** Butyl Parahydroxybenzoate occurs as colorless crystals or white crystalline powder.

It is very soluble in methanol, freely soluble in ethanol (95) and in acetone, and practically insoluble in water.◆

Identification Determine the infrared absorption spectrum of Butyl Parahydroxybenzoate as directed in the potassium bromide disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum or the spectrum of Butyl Parahydroxybenzoate RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

Melting point <*2.60*> 68 – 71°C

Purity **(1)** Clarity and color of solution—Dissolve 1.0 g of Butyl Parahydroxybenzoate in ethanol (95) to make 10 mL: the solution is clear and not more intensely colored than ethanol (95) or the following control solution.

Control solution: To 5.0 mL of Cobalt (II) Chloride CS, 12.0 mL of Iron (III) Chloride CS and 2.0 mL of Copper (II) Sulfate CS add diluted dilute hydrochloric acid (1 in 10) to make 1000 mL.

(2) Acidity—To 2 mL of the solution of Butyl Parahydroxybenzoate obtained in (1) add 3 mL of ethanol (95), add 5 mL of freshly boiled and cooled water and 0.1 mL of bromocresol green-sodium hydroxide-ethanol TS, then add 0.1 mol/L sodium hydroxide VS until the solution shows a blue color: the volume of 0.1 mol/L sodium hydroxide VS used does not exceed 0.1 mL.

(3) Related substances—Dissolve 50.0 mg of Butyl Parahydroxybenzoate in 2.5 mL of methanol, and add the mobile phase to make exactly 50 mL. Pipet 10 mL of this solution, add the mobile phase to make exactly 100 mL, and use this solution as the sample solution. Pipet 1 mL of the sample solution, and add the mobile phase to make exactly 20 mL. Pipet 1 mL of this solution, add the mobile phase to make exactly 10 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine each peak area by the automatic integration method: the peak area of parahydroxybenzoic acid having the relative retention time of about 0.1 to butyl parahydroxybenzoate obtained from the sample solution is not larger than the peak area of butyl parahydroxybenzoate from the standard solution (0.5%). For the area of the peak of parahydroxybenzoic acid multiply the correction factor, 1.4. Furthermore, the area of the peak other than butyl parahydroxybenzoate and parahydroxybenzoic acid from the sample solution is not larger than the peak area of butyl parahydroxybenzoate from the standard solution (0.5%), and the total area of the peaks other than butyl parahydroxybenzoate is not larger than 2 times the peak area of butyl parahydroxybenzoate from the standard solution (1.0%). For this calculation the peak area not larger than 1/5 times the peak area of butyl parahydroxybenzoate from the standard solution is excluded (0.1%).

Operating conditions—

Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay.

Time span of measurement: 1.5 times as long as the retention time of butyl parahydroxybenzoate.

System suitability—

System performance: Proceed as directed in the system suitability in the Assay.

◇Test for required detectability: To exactly 2 mL of the standard solution add the mobile phase to make exactly 10 mL. Confirm that the peak area of butyl parahydroxybenzoate obtained with 10 μL of this solution is equivalent to 14 to 26% of that with 10 μL of the standard solution.◇

◇System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of butyl parahydroxybenzoate is not more than 2.0%.◇

Residue on ignition <*2.44*> Not more than 0.1% (1 g).

Assay Weigh accurately about 50 mg each of Butyl Parahydroxybenzoate and Butyl Parahydroxybenzoate RS, dissolve separately in 2.5 mL each of methanol, and add the mobile phase to make exactly 50 mL. Pipet 10 mL each of these solutions, add the mobile phase to make exactly 100 mL, and use these solutions as the sample solution and the standard solution, respectively. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine the peak areas, A_T and A_S, of butyl parahydroxybenzoate in each solution.

Amount (mg) of butyl parahydroxybenzoate ($C_{11}H_{14}O_3$) $= M_S \times A_T/A_S$

M_S: Amount (mg) of Butyl Parahydroxybenzoate RS taken

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 272 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 35°C.

Mobile phase: A mixture of potassium dihydrogen phos-

phate solution (17 in 2500) and methanol (1:1).

Flow rate: 1.3 mL per minute.

System suitability—

System performance: Dissolve 5 mg each of Butyl Parahydroxybenzoate, propyl parahydroxybenzoate and parahydroxybenzoic acid in the mobile phase to make exactly 100 mL. Pipet 1 mL of this solution, add the mobile phase to make exactly 10 mL, and use this solution as the solution for system suitability test (1). Separately, dissolve 5 mg of isobutyl parahydroxybenzoate in the mobile phase to make exactly 100 mL. Pipet 0.5 mL of this solution, add the standard solution to make exactly 50 mL, and use this solution as the solution for system suitability test (2). When the procedure is run with 10 μL each of the solutions for system suitability test (1) and (2) under the above operating conditions, parahydroxybenzoic acid, propyl parahydroxybenzoate, isobutyl parahydroxybenzoate and butyl parahydroxybenzoate are eluted in this order, the relative retention times of parahydroxybenzoic acid, propyl parahydroxybenzoate and isobutyl parahydoxybenzoate to butyl parahydroxybenzoate are about 0.1, about 0.5 and about 0.9, respectively, the resolution between the peaks of propyl parahydroxybenzoate and butyl parahydroxybenzoate is not less than 5.0, and the resolution between the peaks of isobutyl parahydroxybenzoate and butyl parahydroxybenzoate is not less than 1.5.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of butyl parahydroxybenzoate is not more than 0.85%.

◆**Containers and storage** Containers—Well-closed containers.◆

Croscarmellose Sodium

クロスカルメロースナトリウム

Change the Identification as follows:

Identification (1) Determine the infrared absorption spectrum of Croscarmellose Sodium as directed in the potassium bromide disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers. If there is an absorption at about 1750 cm^{-1}, disregard the absorption.

(2) To 1 g of Croscarmellose Sodium add 100 mL of a solution of methylene blue (1 in 250,000), stir thoroughly, and allow to stand: blue cotton-like precipitates appear.

(3) Dissolve 0.1 g of the residue obtained in the Residue on ignition in 2 mL of water, add 2 mL of potassium carbonate solution (3 in 20), and heat to boiling: no precipitate is formed. To this solution add 4 mL of potassium hexahydroxoantimonate (V) TS, heat to boiling, and cool immediately in ice water, if necessary, rubbing the inside wall of the test tube with a glass rod: a white crystalline precipitate is formed.

Delete the Purity (1) and move up the section number sequentially, and change as follows:

Purity ◆**(1)** Sodium chloride and sodium glycolate—The total amount of sodium chloride and sodium glycolate is not more than 0.5%, calculated on the dried basis.

(i) Sodium chloride: Weigh accurately about 5 g of Croscarmellose Sodium, add 50 mL of water and 5 mL of hydrogen peroxide (30), and heat on a water bath for 20 minutes with occasional stirring. After cooling, add 100 mL of water and 10 mL of nitric acid, and titrate <*2.50*> with 0.1 mol/L silver nitrate VS (potentiometric titration). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L silver nitrate VS
= 5.844 mg of NaCl

(ii) Sodium glycolate: Weigh accurately about 0.5 g of Croscarmellose Sodium, add 2 mL of acetic acid (100) and 5 mL of water, and stir for 15 minutes. Add gradually 50 mL of acetone with stirring, then add 1 g of sodium chloride, stir for 3 minutes, and filter through a filter paper moistened with acetone. Wash the residue thoroughly with 30 mL of acetone, combine the filtrate and washings, add acetone to make exactly 100 mL, and use this solution as the sample stock solution. Separately, dissolve 0.100 g of glycolic acid in water to make exactly 200 mL. Pipet 0.5 mL, 1 mL, 2 mL, 3 mL and 4 mL of this solution, add water to make them exactly 5 mL, then add 5 mL of acetic acid (100) and acetone to make exactly 100 mL, and designate them standard stock solution (1), standard stock solution (2), standard stock solution (3), standard stock solution (4) and standard stock solution (5), respectively. Pipet 2 mL each of the sample stock solution and the standard stock solutions (1), (2), (3), (4) and (5), and heat them in a water bath for 20 minutes to evaporate acetone. After cooling, add exactly 5 mL of 2,7-dihydroxynaphthalene TS, mix, then add 15 mL of 2,7-dihydroxynaphthalene TS, mix, cover the mouth of the vessels with aluminum foil, and heat in a water bath for 20 minutes. After cooling, add sulfuric acid to make exactly 25 mL, mix, and use them as the sample solution, standard solution (1), standard solution (2), standard solution (3), standard solution (4) and standard solution (5), respectively. Separately, to 10 mL of a mixture of water and acetic acid (100) (1:1) add acetone to make exactly 100 mL, and proceed with exactly 2 mL of this solution in the same manner for preparation of the sample solution, and use the solution so obtained as the blank solution. Determine the absorbances, A_T, A_{S1}, A_{S2}, A_{S3}, A_{S4} and A_{S5}, of the sample solution and the standard solutions (1), (2), (3), (4) and (5), respectively, at 540 nm as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, using the blank solution as the control. Determine the amount (g) of glycolic acid, X, in 100 mL of the sample stock solution from the calibration curve obtained with the standard solutions, and calculate the amount of sodium glycolate by the following formula.

Amount (%) of sodium glycolate
$= X/M \times 100 \times 1.289$

M: Amount (g) of sample taken, calculated on the dried basis◆

◆**(2)** Water-soluble substance—Weigh accurately about 10 g of Croscarmellose Sodium, disperse in 800 mL of water by stirring for 1 minute every 10 minutes during 30 minutes. If precipitation is slow, allow to stand another 1 hour. Filter by suction or centrifuge. Weigh accurately the mass of about 150 mL of the filtrate or supernatant liquid. Heat to concentrate this liquid avoiding to dryness, then dry at 105° C for 4 hours, and weigh the mass of the residue accurately. Calculate the amount of the water-soluble substance by the following formula: not less than 1.0% and not more than 10.0%.

Amount (%) of water-soluble substance
$= 100 \times M_3 \times (800 + M_1)/(M_1 \times M_2)$

M_1: Amount (g) of sample taken, calculated on the dried basis
M_2: Amount (g) of the filtrate or supernatant liquid of about 150 mL
M_3: Amount (g) of the residue◆

Change the Residue on ignition, Containers and storage as follows:

Residue on ignition ⟨*2.44*⟩ 14.0 – 28.0% (1 g calculated on the dried basis).

◆**Containers and storage** Containers—Tight containers.◆

Cefoperazone Sodium and Sulbactam Sodium for Injection

注射用セフォペラゾンナトリウム·スルバクタムナトリウム

Change the description of Uniformity of dosage units as follows:

Uniformity of dosage units ⟨*6.02*⟩ It meets the requirement of the Mass variation test (*T*: Being specified separately when the drug is granted approval based on the Law.).

Change the following as follows:

Powdered Cellulose

粉末セルロース

[*9004-34-6*, Cellulose]

This monograph is harmonized with the European Pharmacopoeia and the U.S. Pharmacopeia.

The corresponding part of the attributes/provisions which are agreed as non-harmonized within the scope of the harmonization is marked with symbols (◆ ◆), and the corresponding parts which are agreed as the JP local requirement other than the scope of the harmonization are marked with symbols (◇ ◇).

Information on the harmonization with the European Pharmacopoeia and the U.S. Pharmacopeia is available on the website of the Pharmaceuticals and Medical Devices Agency.

Powdered Cellulose is a purified, mechanically disintegrated alpha cellulose obtained as a pulp, ◇after partial hydrolysis as occasion demands◇, from fibrous plant materials.

◆The label indicates the mean degree of polymerization value with a range.◆

◆**Description** Powdered Cellulose occurs as a white powder.

It is practically insoluble in water, in ethanol (95) and in diethyl ether.◆

Identification (1) Dissolve 20 g of zinc chloride and 6.5 g of potassium iodide in 10.5 mL of water, add 0.5 g of iodine, and shake for 15 minutes. Place about 10 mg of Powdered Cellulose on a watch glass, and disperse in 2 mL of this solution: the substance develops a blue-violet color.

◇**(2)** Mix 30 g of Powdered Cellulose with 270 mL of water in a high-speed (18,000 revolutions per minute or more) blender for 5 minutes, transfer 100 mL of the dispersion to a 100-mL graduated cylinder, and allow to stand for 1 hour: a supernatant liquid appears above the layer of the cellulose.◇

(3) Transfer 0.25 g of Powdered Cellulose, accurately weighed, to a 125-mL conical flask, add exactly 25 mL each of water and 1 mol/L cupriethylenediamine TS, and proceed as directed in the Identification (3) under Microcrystalline Cellulose. The mean degree of polymerization, P, is not less than 440 ◆and is within the labeled specification.◆

pH ⟨*2.54*⟩ Mix 10 g of Powdered Cellulose with 90 mL of water, and allow to stand for 1 hour with occasional stirring: the pH of the supernatant liquid is between 5.0 and 7.5.

Purity (1) Water-soluble substances—Shake 6.0 g of Powdered Cellulose with 90 mL of recently boiled and cooled water, and allow to stand for 10 minutes with occasional shaking. Filter, with the aid of vacuum through a filter paper, discard the first 10 mL of the filtrate, and pass the subsequent filtrate through the same filter, if necessary, to obtain a clear filtrate. Evaporate a 15.0-mL portion of the filtrate in a tared evaporating dish to dryness without charring, dry at 105°C for 1 hour, and weigh after allowing to cool in a desiccator: the difference between the mass of the residue and the mass obtained from a blank determination does not exceed 15.0 mg (1.5%).

(2) Diethyl ether-soluble substances—Place 10.0 g of

Powdered Cellulose in a column having an internal diameter of about 20 mm, and pass 50 mL of peroxide-free diethyl ether through the column. Evaporate the eluate to dryness in a previously dried and tared evaporation dish. Dry the residue at 105°C for 30 minutes, and weigh after allowing to cool in a desiccator: the difference between the mass of the residue and the mass obtained from a blank determination does not exceed 15.0 mg (0.15%).

Loss on drying ⟨*2.41*⟩ Not more than 6.5% (1 g, 105°C, 3 hours).

Residue on ignition ⟨*2.44*⟩ Not more than 0.3% (1 g calculated on the dried basis).

◆**Microbial limit** ⟨*4.05*⟩ The acceptance criteria of TAMC and TYMC are 10^3 CFU/g and 10^2 CFU/g, respectively. *Escherichia coli*, *Salmonella*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* are not observed.◆

◆**Containers and storage** Containers—Tight containers.◆

Enviomycin Sulfate

エンビオマイシン硫酸塩

Change the Content ratio of the active principle as follows:

Content ratio of the active principle Dissolve about 50 mg of Enviomycin Sulfate in water to make 100 mL, and use this solution as the sample solution. Perform the test with 5 μL of the sample solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, and determine the peak areas, A_{T1} and A_{T2}, of tuberactinomycin N and tuberactinomycin O, having the relative retention time, about 1.2, to tuberactinomycin N, by the automatic integration method: $A_{T2}/(A_{T1} + A_{T2})$ is between 0.090 and 0.150.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (3 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: A mixture of water and trifluoroacetic acid (1000:1).

Flow rate: Adjust so that the retention time of tuberactinomycin N is about 15 minutes.

System suitability—

System performance: When the procedure is run with 5 μL of the sample solution under the above operating conditions, tuberactinomycin N and tuberactinomycin O are eluted in this order with the resolution between these peaks being not less than 3.

System repeatability: When the test is repeated 6 times with 5 μL of the sample solution under the above operating conditions, the relative standard deviation of the peak area of tuberactinomycin N is not more than 2.0%.

Epoetin Beta (Genetical Recombination)

エポエチン ベータ(遺伝子組換え)

Change the Identification (1) as follows:

Identification (1) Desalt an appropriate volume each of Epoetin Beta (Genetical Recombination) and Epoetin Beta RS by a suitable method, add water if necessary, to make solutions so that each mL contains about 1 mg of each protein, and use these solutions as the sample solution and the standard solution, respectively. When perform a capillary electrophoresis with the sample solution and standard solution according to the following conditions, the relative migration time of each peak to the peak of the electroosmotic flow obtained from both solutions is the same and their electropherograms are similar to each other.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 214 nm).

Column: A silica capillary tube 50 μm in inside diameter and about 110 cm in length (about 100 cm in effective length, washed with a suitable alkaline solution and pretreated with the electrolyte solution).

Electrolyte solution: Dissolve 0.58 g of sodium chloride, 1.79 g of tricine and 0.82 g of anhydrous sodium acetate in water to make 100 mL, and use this solution as the electrolyte stock solution. Separately, dissolve 42 g of urea in 50 mL of water, add 10 mL of the electrolyte stock solution and 250 μL of 1 mol/L 1,4-diaminobutane solution, add water to make 100 mL, adjust to pH 5.6 with diluted acetic anhydride (1 in 20), and filter through a membrane filter with a pore size of 0.45 μm.

Running temperature: A constant temperature of about 35°C.

Running conditions: Migration voltage (applied voltage of about 17 kV), migration time (100 minutes).

Injection of sample and standard solutions: 15 seconds (pressurization: 10.3 kPa).

Time span of measurement: For 100 minutes after injection.

System suitability—

System performance: When the procedure is run with the standard solution under the above operating conditions, more than 4 major peaks of epoetin beta are detected, and the resolution between the first and second eluted major peaks is not less than 0.8.

System repeatability: When the test is repeated 3 times with the standard solution under the above operating conditions, the relative standard deviation of the relative migration time of the first eluted major peak to the peak of the electroosmotic flow detected before the peak of epoetin beta

is not more than 2%.

Ethanol

エタノール

Change the description of the international harmonization at the beginning of the text, containers and storage, and shelf life as follows:

This monograph is harmonized with the European Pharmacopoeia and the U.S. Pharmacopeia.

The corresponding part of the attributes/provisions which are agreed as non-harmonized within the scope of the harmonization is marked with symbols (◆ ◆), and the corresponding parts which are agreed as the JP local requirement other than the scope of the harmonization are marked with symbols (◇ ◇).

Information on the harmonization with the European Pharmacopoeia and the U.S. Pharmacopeia is available on the website of the Pharmaceuticals and Medical Devices Agency.

Containers and storage ◇Containers—Tight containers.◇
Storage—Without exposure to light.

◇**Shelf life** In not glass containers: Unless otherwise specified, 24 months after preparation.◇

Anhydrous Ethanol

無水エタノール

Change the description of the international harmonization at the beginning of the text, containers and storage, and shelf life as follows:

This monograph is harmonized with the European Pharmacopoeia and the U.S. Pharmacopeia.

The corresponding part of the attributes/provisions which are agreed as non-harmonized within the scope of the harmonization is marked with symbols (◆ ◆), and the corresponding parts which are agreed as the JP local requirement other than the scope of the harmonization are marked with symbols (◇ ◇).

Information on the harmonization with the European Pharmacopoeia and the U.S. Pharmacopeia is available on the website of the Pharmaceuticals and Medical Devices Agency.

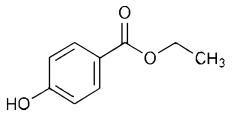
Containers and storage ◇Containers—Tight containers.◇
Storage—Without exposure to light.

◇**Shelf life** In not glass containers: Unless otherwise specified, 24 months after preparation.◇

Change the following as follows:

Ethyl Parahydroxybenzoate

パラオキシ安息香酸エチル



$C_9H_{10}O_3$: 166.17
Ethyl 4-hydroxybenzoate
[*120-47-8*]

This monograph is harmonized with the European Pharmacopoeia and the U.S. Pharmacopeia.

The corresponding part of the attributes/provisions which are agreed as non-harmonized within the scope of the harmonization is marked with symbols (◆ ◆), and the corresponding parts which are agreed as the JP local requirement other than the scope of the harmonization are marked with symbols (◇ ◇).

Information on the harmonization with the European Pharmacopoeia and the U.S. Pharmacopeia is available on the website of the Pharmaceuticals and Medical Devices Agency.

Ethyl Parahydroxybenzoate contains not less than 98.0% and not more than 102.0% of ethyl parahydroxybenzoate ($C_9H_{10}O_3$).

◆**Description** Ethyl Parahydroxybenzoate occurs as colorless crystals or a white, crystalline powder.

It is freely soluble in methanol, in ethanol (95) and in acetone, and very slightly soluble in water.◆

Identification Determine the infrared absorption spectrum of Ethyl Parahydroxybenzoate as directed in the potassium bromide disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum or the spectrum of Ethyl Parahydroxybenzoate RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

Melting point <*2.60*> 115 – 118°C

Purity **(1)** Clarity and color of solution—Dissolve 1.0 g of Ethyl Parahydroxybenzoate in ethanol (95) to make 10 mL: the solution is clear and not more intensely colored than ethanol (95) or the following control solution.

Control solution: To 5.0 mL of Cobalt (II) Chloride CS, 12.0 mL of Iron (III) Chloride CS and 2.0 mL of Copper (II) Sulfate CS add diluted dilute hydrochloric acid (1 in 10) to make 1000 mL.

(2) Acidity—To 2 mL of the solution of Ethyl Parahydroxybenzoate obtained in (1) add 3 mL of ethanol (95), add 5 mL of freshly boiled and cooled water and 0.1 mL of bromocresol green-sodium hydroxide-ethanol TS, then add 0.1 mol/L sodium hydroxide VS until the solution shows a blue color: the volume of 0.1 mol/L sodium hydroxide VS used does not exceed 0.1 mL.

(3) Related substances—Dissolve 50.0 mg of Ethyl Parahydroxybenzoate in 2.5 mL of methanol, and add the mobile phase to make exactly 50 mL. Pipet 10 mL of this solution, add the mobile phase to make exactly 100 mL, and use this solution as the sample solution. Pipet 1 mL of the sample solution, and add the mobile phase to make exactly 20 mL. Pipet 1 mL of this solution, add the mobile phase to make exactly 10 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine each peak area by the automatic integration method: the peak area of parahydroxybenzoic acid having the relative retention time of about 0.5 to ethyl parahydroxybenzoate obtained from the sample solution is not larger than the peak area of ethyl parahydroxybenzoate from the standard solution (0.5%). For the peak area of parahydroxybenzoic acid, multiply the correction factor, 1.4. Furthermore, the area of the peak other than ethyl parahydroxybenzoate and parahydroxybenzoic acid from the sample solution is not larger than the peak area of ethyl parahydroxybenzoate from the standard solution (0.5%), and the total area of the peaks other than ethyl parahydroxybenzoate is not larger than 2 times the peak area of ethyl parahydroxybenzoate from the standard solution (1.0%). For this calculation the peak area not larger than 1/5 times the peak area of ethyl parahydroxybenzoate from the standard solution is excluded (0.1%).

Operating conditions—

Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay.

Time span of measurement: 4 times as long as the retention time of ethyl parahydroxybenzoate.

System suitability—

System performance: Proceed as directed in the system suitability in the Assay.

◇Test for required detectability: To exactly 2 mL of the standard solution add the mobile phase to make exactly 10 mL. Confirm that the peak area of ethyl parahydroxybenzoate obtained with 10 μL of this solution is equivalent to 14 to 26% of that with 10 μL of the standard solution.◇

◇System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of ethyl parahydroxybenzoate is not more than 2.0%.◇

Residue on ignition <*2.44*> Not more than 0.1% (1 g).

Assay Weigh accurately about 50 mg each of Ethyl Parahydroxybenzoate and Ethyl Parahydroxybenzoate RS, dissolve separately in 2.5 mL each of methanol, and add the mobile phase to make exactly 50 mL. Pipet 10 mL each of these solutions, add the mobile phase to make exactly 100 mL, and use these solutions as the sample solution and the standard solution, respectively. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine the peak areas, A_T and A_S, of ethyl parahydroxybenzoate in each solution.

Amount (mg) of ethyl parahydroxybenzoate ($C_9H_{10}O_3$) $= M_S \times A_T/A_S$

M_S: Amount (mg) of Ethyl Parahydroxybenzoate RS taken

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 272 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

◇Column temperature: A constant temperature of about 35°C.◇

Mobile phase: A mixture of methanol and potassium dihydrogen phosphate solution (17 in 2500) (13:7).

Flow rate: 1.3 mL per minute.

System suitability—

System performance: Dissolve 5 mg each of Ethyl Parahydroxybenzoate, methyl parahydroxybenzoate and parahydroxybenzoic acid in the mobile phase to make exactly 100 mL. Pipet 1 mL of this solution, and add the mobile phase to make exactly 10 mL. When the procedure is run with 10 μL of this solution under the above operating conditions, parahydroxybenzoic acid, methyl parahydroxybenzoate and ethyl parahydroxybenzoate are eluted in this order, the relative retention times of parahydroxybenzoic acid and methyl parahydroxybenzoate to ethyl parahydroxybenzoate are about 0.5 and about 0.8, respectively, and the resolution between the peaks of methyl parahydroxybenzoate and ethyl parahydroxybenzoate is not less than 2.0.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of ethyl parahydroxybenzoate is not more than 0.85%.

◆**Containers and storage** Containers—Well-closed containers.◆

Formoterol Fumarate Hydrate

ホルモテロールフマル酸塩水和物

Change the Chemical name and Purity as follows:

$(C_{19}H_{24}N_2O_4)_2.C_4H_4O_4.2H_2O$: 840.91

N-(2-Hydroxy-5-{(1*RS*)-1-hydroxy-2-[(2*RS*)-1-(4-methoxyphenyl)propan-2-ylamino]ethyl}phenyl)formamide hemifumarate monohydrate

Purity (1) Related Substances—Dissolve 20 mg of Formoterol Fumarate Hydrate in the diluting solution to make 100 mL, and use this solution as the sample solution. Perform the test with 20 μL of the sample solution as directed under Liquid Chromatography <*2.01*> according to the fol-

lowing conditions. Determine each peak area by the automatic integration method, and calculate their amounts by the area percentage method: the amount of the peak of the related substance A, having the relative retention time of about 0.5 to formoterol, is not more than 0.3%, the amounts of the peaks of the related substances B, C, D and F, having the relative retention times of about 0.7, about 1.2, about 1.3 and about 2.0, are not more than 0.2%, respectively, the amount of the peak of the related substance E, having the relative retention time of about 1.8, is not more than 0.1%, and the amount of the peak other than formoterol and the peaks mentioned above is not more than 0.1%. Furthermore, the total amount of the peaks other than formoterol is not more than 0.5%. For the peak area of the related substance A, multiply the correction factor, 1.75.

Diluting solution: Dissolve 6.9 g of sodium dihydrogen phosphate dihydrate and 0.8 g of anhydrous disodium hydrogen phosphate in water to make 1000 mL, and adjust to pH 6.0 with 0.5 mol/L disodium hydrogen phosphate TS or diluted phosphoric acid (27 in 400). To 21 volumes of this solution add 4 volumes of acetonitrile.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 214 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 22°C.

Mobile phase A: Dissolve 4.2 g of sodium dihydrogen phosphate dihydrate and 0.35 g of phosphoric acid in water to make 1000 mL, and adjust to pH 3.1 with a solution prepared by dissolving 156 g of sodium dihydrogen phosphate dihydrate in water to make 1000 mL or diluted phosphoric acid (27 in 400).

Mobile phase B: Acetonitrile for liquid chromatography.

Flowing of mobile phase: Control the gradient by mixing the mobile phases A and B as directed in the following table.

Time after injection of sample (min)	Mobile phase A (vol%)	Mobile phase B (vol%)
0 – 10	84	16
10 – 37	84 → 30	16 → 70

Flow rate: 1.0 mL per minute (the retention time of formoterol is about 10 minutes).

Time span of measurement: For 37 minutes after injection, beginning after the peak of fumaric acid.

System suitability—

Test for required detectability: Pipet 1 mL of the sample solution, and add the diluting solution to make exactly 100 mL. Pipet 1 mL of this solution, add the diluting solution to make exactly 20 mL. When the procedure is run with 20 μL of this solution under the above operating conditions, the SN ratio of the peak of formoterol is not less than 10.

System performance: When the procedure is run with 20 μL of the sample solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of formoterol are not less than 2000 and not more than 3.0, respectively.

(2) Diastereomer—Dissolve 5 mg of Formoterol Fumarate Hydrate in water to make 50 mL, and use this solution as the sample solution. Perform the test with 20 μL of the sample solution as directed under Liquid Chromatography <*2.01*> according to the following conditions. Determine the peak areas, A_f and A_d, of formoterol and the related substance I (diastereomer), having the relative retention time of about 1.2 to formoterol, in the sample solution by the automatic integration method, and calculate the amount of the diastereomer by the following equation: not more than 0.3%.

$$\text{Amount (\%) of diastereomer} = A_d/(A_d + A_f) \times 100$$

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 225 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized polyvinyl alcohol gel polymer for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 22°C.

Mobile phase: Dissolve 5.3 g of potassium phosphate trihydrate in water to make 1000 mL, and adjust to pH 12.0 with a solution of potassium hydroxide (281 in 1000) or phosphoric acid. To 22 volumes of this solution add 3 volumes of acetonitrile for liquid chromatography.

Flow rate: 0.5 mL per minute (the retention time of formoterol is about 22 minutes).

System suitability—

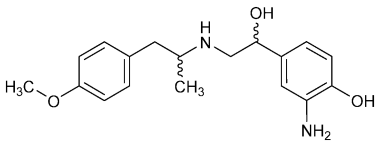
Test for required detectability: Pipet 1 mL of the sample solution, and add water to make exactly 20 mL. Pipet 1 mL of this solution, and add water to make exactly 25 mL. When the procedure is run with 20 μL of this solution under the above operating conditions, the SN ratio of the peak of formoterol is not less than 10.

System performance: When the procedure is run with 20 μL of the sample solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of formoterol are not less than 4300 and not more than 1.7, respectively.

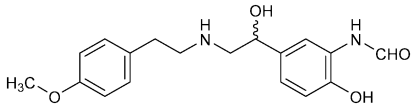
Add the following next to the Containers and storage:

Others

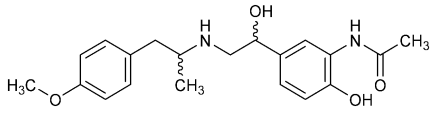
Related substance A:
2-Amino-4-{1-hydroxy-2-[1-(4-methoxyphenyl)propan-2-ylamino]ethyl}phenol



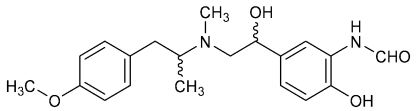
Related substance B:
N-(2-Hydroxy-5-{1-hydroxy-2-[2-(4-methoxyphenyl)ethylamino]ethyl}phenyl)formamide



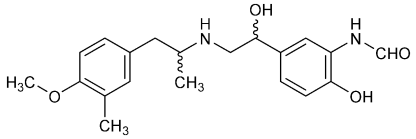
Related substance C:
N-(2-Hydroxy-5-{1-hydroxy-2-[1-(4-methoxyphenyl)propan-2-ylamino]ethyl}phenyl)acetamide



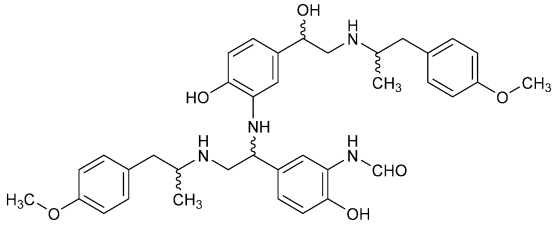
Related substance D:
N-(2-Hydroxy-5-{1-hydroxy-2-[1-(4-methoxyphenyl)propan-2-ylmethylamino]ethyl}phenyl)formamide



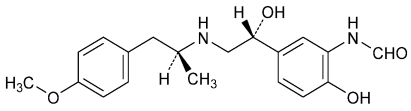
Related substance E:
N-(2-Hydroxy-5-{1-hydroxy-2-[1-(4-methoxy-3-methylphenyl)propan-2-ylamino]ethyl}phenyl)formamide



Related substance F:
N-(2-Hydroxy-5-{1-(2-hydroxy-5-{1-hydroxy-2-[1-(4-methoxyphenyl)propan-2-ylamino]ethyl}phenyl)amino-2-[1-(4-methoxyphenyl)propan-2-ylamino]ethyl}phenyl)formamide



Related substance I (diastereomer):
N-(2-Hydroxy-5-{(1*RS*)-1-hydroxy-2-[(2*SR*)-1-(4-methoxyphenyl)propan-2-ylamino]ethyl}phenyl)formamide



Glyceryl Monostearate

モノステアリン酸グリセリン

Delete the Identification (1), and change as follows:

Identification Dissolve 0.1 g of Glyceryl Monostearate in 2 mL of ethanol (95) by warming, heat with 5 mL of dilute sulfuric acid in a water bath for 30 minutes, and cool; a white to yellow solid is produced. This separated solid dissolves when shaken with 3 mL of diethyl ether.

Hypromellose Phthalate

ヒプロメロースフタル酸エステル

Change the preamble concerning the harmonization to the beginning of the text, and change the Description and the Viscosity:

This monograph is harmonized with the European Pharmacopoeia and the U.S. Pharmacopeia.

The corresponding part of the attributes/provisions which are agreed as non-harmonized within the scope of the harmonization is marked with symbols (◆ ◆), and the corresponding parts which are agreed as the JP local requirement other than the scope of the harmonization are marked with symbols (◇ ◇).

Information on the harmonization with the European Pharmacopoeia and the U.S. Pharmacopeia is available on the website of the Pharmaceuticals and Medical Devices Agency.

◆**Description** Hypromellose Phthalate occurs as white, powder or granules.

It is practically insoluble in water, in acetonitrile and in ethanol (99.5).

It becomes a viscous liquid when a mixture of methanol and dichloromethane (1:1 in mass ratio) or a mixture of ethanol (99.5) and acetone (1:1) is added.

It dissolves in sodium hydroxide TS.◆

Viscosity ⟨*2.53*⟩ To 10 g of Hypromellose Phthalate, previously dried at 105°C for 1 hour, add 90 g of a mixture of methanol and dichloromethane (1:1 in mass ratio), stir, and shake to dissolve. Determine the viscosity at 20 ± 0.1°C as directed in Method 1 under Viscosity Determination: the viscosity is not less than 80% and not more than 120% of the labeled unit.

Delete the Purity (2) and move up the after section, and change as follows:

Purity

(2) Phthalic acid—Weigh accurately about 0.2 g of Hypromellose Phthalate, add about 50 mL of acetonitrile to dissolve partially by sonicating, add 10 mL of water, and dissolve further by sonicating. After cooling, add acetonitrile to make exactly 100 mL, and use this solution as the sample solution. Separately, weigh accurately about 12.5 mg of phthalic acid, dissolve in about 125 mL of acetonitrile by mixing, add 25 mL of water, then add acetonitrile to make exactly 250 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine the peak areas, A_T and A_S, of phthalic acid in each solution: amount of phthalic acid ($C_8H_6O_4$: 166.13) is not more than 1.0%.

Amount (%) of phthalic acid = $M_S/M_T \times A_T/A_S \times 40$

M_S: Amount (mg) of phthalic acid taken
M_T: Amount (mg) of Hypromellose Phthalate taken, calculated on the anhydrous basis

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 235 nm).

Column: A stainless steel column about 4.6 mm in inside diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (3 to 10 μm in particle diameter).

Column temperature: A constant temperature of about 20°C.

Mobile phase: A mixture of 0.1% trifluoroacetic acid and acetonitrile (9:1).

Flow rate: About 2.0 mL per minute.

System suitability—

◇System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of phthalic acid are not less than 2500 and not more than 1.5, respectively.◇

System repeatability: When repeat the test 5 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of phthalic acid is not more than 1.0%.

Imipenem and Cilastatin Sodium for Injection

注射用イミペネム·シラスタチンナトリウム

Change the description of Uniformity of dosage units as follows:

Uniformity of dosage units <*6.02*> Perform the test according to the following method: it meets the requirement of the Content uniformity test (*T*: Being specified separately when the drug is granted approval based on the Law.).

Dissolve the total amount of the content of 1 Imipenem and Cilastatin Sodium for Injection in isotonic sodium chloride solution to make exactly 100 mL. Pipet *V* mL of this solution, equivalent to about 25 mg (potency) of Imipenem Hydrate, add 0.1 mol/L 3-(*N*-morpholino) propanesulfonic acid buffer solution (pH 7.0) to make exactly 50 mL, and use this solution as the sample solution. Then, proceed as directed in the Assay.

Amount [mg (potency)] of imipenem ($C_{12}H_{17}N_3O_4S$) = $M_{SI} \times A_{TI}/A_{SI} \times 100/V$

Amount (mg) of cilastatin ($C_{16}H_{26}N_2O_5S$) = $M_{SC} \times A_{TC}/A_{SC} \times 100/V \times 0.955$

M_{SI}: Amount [mg (potency)] of Imipenem RS taken
M_{SC}: Amount (mg) of cilastatin ammonium for assay taken, calculated on anhydrous and ethanol-free basis

Insulin Human (Genetical Recombination)

インスリン　ヒト(遺伝子組換え)

Change the Identification and Assay as follows:

Identification Dissolve a suitable amount of Insulin Human (Genetical Recombination) in 0.01 mol/L hydrochloric acid TS to make a solution so that each mL contains 2.0 mg, and use this solution as the sample stock solution. Separately, dissolve Insulin Human RS in 0.01 mol/L hydrochloric acid TS to make a solution so that each mL contains 2.0 mg, and use this solution as the standard stock solution. Transfer 500 μL each of these solutions into a clean test tube, add 2.0 mL of HEPES buffer solution (pH 7.5) and 400 μL of V8-protease TS, incubate at 25°C for 6 hours, then add 2.9 mL of ammonium sulfate buffer solution to stop the reaction, and use these solutions as the standard solution and the sample solution. Perform the test with 50 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and compare the chromatograms obtained from these solutions: a similar peak is observed at the same retention time in the both chromatograms.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 214 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 10 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (3 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase A: A mixture of water, ammonium sulfate

buffer solution and acetonitrile (7:2:1).

Mobile phase B: A mixture of water, acetonitrile and ammonium sulfate buffer solution (2:2:1).

Flowing of mobile phase: Change the mixing ratio of the mobile phase A and B linearly from 9:1 to 3:7 in 60 minutes after sample injection, further change to 0:10 linearly in 5 minutes, and then flow the mobile phase B only for 5 minutes.

Flow rate: 1.0 mL per minute.

System suitability—

System performance: When the procedure is run with 50 μL of the standard solution under the above operating conditions, the symmetry factor of the two larger peaks which appear next to the first peak just after the solvent peak are not more than 1.5 respectively, and the resolution between these peaks is not less than 3.4.

Assay Perform this procedure quickly. Weigh accurately about 7.5 mg of Insulin Human (Genetical Recombination), dissolve in 0.01 mol/L hydrochloric acid TS to make exactly 5 mL, and use this solution as the sample solution. Separately, dissolve exactly Insulin Human RS in 0.01 mol/L hydrochloric acid TS to make a solution so that each mL contains about 40 Insulin Units, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine the peak areas of human insulin, A_{TI} and A_{SI}, and the peak areas of the desamido substance having the relative retention time of 1.3 to human insulin, A_{TD} and A_{SD}, respectively, of these solutions.

Amount (Insulin Unit/mg) of human insulin ($C_{257}H_{383}N_{65}O_{77}S_6$)
$= M_S/M_T \times (A_{TI} + A_{TD})/(A_{SI} + A_{SD}) \times 5$

M_T: Amount (mg) of Insulin Human (Genetical Recombination) taken, calculated on the dried basis

M_S: Amount (Insulin Unit) of insulin human in 1 mL of the standard solution

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 214 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: A mixture of phosphoric acid-sodium sulfate buffer solution (pH 2.3) and acetonitrile for liquid chromatography (3:1). Adjust the mixing ratio of the component of the mobile phase so that the retention time of human insulin is between 10 minutes and 17 minutes.

Flow rate: 1.0 mL per minute.

System suitability—

System performance: When the procedure is run with 20 μL of human insulin desamido substance-containing TS under the above operating conditions, human insulin and human insulin desamido substance are eluted in this order with the resolution between these peaks being not less than 2.0, and the symmetry factor of the peak of human insulin is not more than 1.8.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak areas of human insulin is not more than 1.6%.

Insulin Human (Genetical Recombination) Injection

インスリン　ヒト(遺伝子組換え)注射液

Change the Assay as follows:

Assay To exactly 10 mL of Insulin Human (Genetical Recombination) Injection add exactly 40 μL of 6 mol/L hydrochloric acid TS. Pipet 2 mL of this solution, add 0.01 mol/L hydrochloric acid TS to make exactly 5 mL, and use this solution as the sample solution. Then, proceed as directed in the Assay under Insulin Human (Genetical Recombination).

Amount (Insulin Unit) of human insulin ($C_{257}H_{383}N_{65}O_{77}S_6$) in 1 mL
$= M_S \times (A_{TI} + A_{TD})/(A_{SI} + A_{SD}) \times 1.004 \times 5/2$

M_S: Amount (Insulin Unit) of insulin human in 1 mL of the standard solution

Isophane Insulin Human (Genetical Recombination) Injectable Aqueous Suspension

イソフェンインスリン　ヒト(遺伝子組換え)水性懸濁注射液

Change the Purity (2) and Assay (1) as follows:

Purity

(2) Dissolved insulin human—Centrifuge Isophane Insulin Human (Genetical Recombination) Injectable Aqueous Suspension, and use the supernatant liquid as the sample solution. Separately, dissolve exactly Insulin Human RS in 0.01 mol/L hydrochloric acid TS to make a solution so that each mL contains about 1.0 Insulin Units, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions. Determine the peak areas, A_T and A_S, of insulin human by the automatic integration method, and calculate the amount of dissolved insulin human by the following equation: not more than 0.5 Insulin Units per mL.

Amount (mg) of dissolved insulin human' (Insulin Unit/mL)
$= M_S \times A_T/A_S$

M_S: Amount (Insulin Unit) of insulin human in 1 mL of the standard solution

Operating conditions—

Proceed as directed in the operating conditions in the Assay (1).

System suitability—

System performance: When the procedure is run with 20 μL of insulin human desamido substance-containing TS under the above operating conditions, insulin human and insulin human desamido substance are eluted in this order with the resolution between these peaks being not less than 2.0, and the symmetry factor of the peak of insulin human is not more than 1.6.

System repeatability: When the test is repeated 4 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of insulin human is not more than 6.0%.

Assay (1) Insulin human—Pipet 10 mL of gently shaken Isophane Insulin Human (Genetical Recombination) Injectable Aqueous Suspension, and add exactly 40 μL of 6 mol/L hydrochloric acid TS. Pipet 2 mL of this solution, add 0.01 mol/L hydrochloric acid TS to make exactly 5 mL, and use this solution as the sample solution. Then, proceed as directed in the Assay under Insulin Human (Genetical Recombination).

Amount (Insulin Unit) of insulin human ($C_{257}H_{383}N_{65}O_{77}S_6$) in 1 mL
$= M_S \times (A_{TI} + A_{TD})/(A_{SI} + A_{SD}) \times 1.004 \times 5/2$

M_S: Amount (Insulin Unit) of insulin human in 1 mL of the standard solution

Biphasic Isophane Insulin Human (Genetical Recombination) Injectable Aqueous Suspension

二相性イソフェンインスリン　ヒト(遺伝子組換え)水性懸濁注射液

Change the Assay (1) as follows:

Assay (1) Insulin human—Pipet 10 mL of gently shaken Biphasic Isophane Insulin Human (Genetical Recombination) Injectable Aqueous Suspension, and add exactly 40 μL of 6 mol/L hydrochloric acid TS. Pipet 2 mL of this solution, add 0.01 mol/L hydrochloric acid TS to make exactly 5 mL, and use this solution as the sample solution. Then, proceed as directed in the Assay under Insulin Human (Genetical Recombination).

Amount (Insulin Unit) of insulin human ($C_{257}H_{383}N_{65}O_{77}S_6$) in 1 mL
$= M_S \times (A_{TI} + A_{TD})/(A_{SI} + A_{SD}) \times 1.004 \times 5/2$

M_S: Amount (Insulin Unit) of insulin human in 1 mL of the standard solution

Change the following as follows:

Magnesium Stearate

ステアリン酸マグネシウム

This monograph is harmonized with the European Pharmacopoeia and the U.S. Pharmacopeia.

The corresponding part of the attributes/provisions which are agreed as non-harmonized within the scope of the harmonization is marked with symbols (◆ ◆), and the corresponding parts which are agreed as the JP local requirement other than the scope of the harmonization are marked with symbols (◇ ◇).

Information on the harmonization with the European Pharmacopoeia and the U. S. Pharmacopeia is available on the website of the Pharmaceuticals and Medical Devices Agency.

Magnesium Stearate is a compound of magnesium with a mixture of solid fatty acids, and consists chiefly of variable proportions of magnesium stearate and magnesium palmitate obtained from sources of vegetable or animal origin.

It contains not less than 4.0% and not more than 5.0% of magnesium (Mg: 24.31), calculated on the dried basis.

◆**Description** Magnesium Stearate occurs as a white, light, bulky powder.

It is smooth to the touch and sticky to the skin. It has no odor or a faint, characteristic odor.

It is practically insoluble in water and in ethanol (99.5).◆

Identification Mix 5.0 g of Magnesium Stearate with 50 mL of peroxide-free diethyl ether, 20 mL of dilute nitric acid, and 20 mL of water in a round-bottom flask, and heat to dissolve completely under a reflux condenser. After cooling, transfer the contents of the flask to a separator, shake, allow the layers to separate, and transfer the aqueous layer to a flask. Extract the diethyl ether layer with two 4-mL portions of water, and combine these extracts to the main aqueous extract. After washing the combined aqueous extract with 15 mL of peroxide-free diethyl ether, transfer to a 50-mL volumetric flask, add water to make 50 mL, and use this solution as the sample solution. To 1 mL of the sample solution add 1 mL of ammonia TS: A white precipitate is formed that dissolves on addition of 1 mL of ammonium chloride TS. By further addition of 1 mL of a solution of disodium hydrogen phosphate dodecahydrate (3 in 25) a white crystalline precipitate is formed.

Purity (1) Acidity or alkalinity—Heat 1.0 g of Magnesium Stearate in 20 mL of freshly boiled and cooled water on

a water bath for 1 minute while shaking, cool, and filter. Add 0.05 mL of bromothymol blue TS to 10 mL of the filtrate: not more than 0.05 mL of 0.1 mol/L hydrochloric acid VS or 0.1 mol/L sodium hydroxide VS is required to change the color of the solution.

(2) Chloride <*1.03*>—To 10.0 mL of the sample solution obtained in Identification add 1 mL of dilute nitric acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution with 1.4 mL of 0.02 mol/L hydrochloric acid VS (not more than 0.1%).

(3) Sulfate <*1.14*>—Perform the test with 6.0 mL of the sample solution obtained in Identification. Prepare the control solution with 3.0 mL of 0.02 mol/L sulfuric acid VS. To the test and control solutions add 3 mL of barium chloride TS (not more than 1.0%).

Loss on drying <*2.41*> Not more than 6.0% (2 g, 105°C, constant mass).

◆**Microbial limit** <*4.05*> The acceptance criteria of TAMC and TYMC are 10^3 CFU/g and 5×10^2 CFU/g, respectively. *Salmonella* and *Escherichia coli* are not observed.◆

Relative content of stearic acid and palmitic acid Transfer 0.10 g of Magnesium Stearate to a small conical flask fitted with a reflux condenser. Add 5.0 mL of boron trifluoride-methanol TS, mix, and reflux for 10 minutes to dissolve the solids. Add 4 mL of heptane through the condenser, and reflux for 10 minutes. After cooling, add 20 mL of saturated sodium chloride solution, shake, and allow the layers to separate. Pass the heptane layer through about 0.1 g of anhydrous sodium sulfate, previously washed with heptane, into another flask. Transfer 1.0 mL of this solution to a 10-mL volumetric flask, dilute with heptane to volume, and use this solution as the sample solution. Perform the test with 1 μL of the sample solution as directed under Gas chromatography <*2.02*> according to the following conditions, and determine the area, A, of the methyl stearate peak and the sum of the areas, B, of all of the fatty acid ester peaks. Calculate the percentage of stearic acid in the fatty acid fraction of Magnesium Stearate by the following formula.

$$\text{Content (\%) of stearic acid} = A/B \times 100$$

Similarly, calculate the percentage of palmitic acid in the portion of Magnesium Stearate taken. The methyl stearate peak, and the sum of the stearate and palmitate peaks are not less than 40% and not less than 90% of the total area of all fatty acid ester peaks, respectively.

Operating conditions—

Detector: A hydrogen flame-ionization detector.

Column: A fused silica capillary column 0.32 mm in inside diameter and 30 m in length, the inside coated with a 0.5-μm layer of polyethylene glycol 15000-diepoxide for gas chromatography.

Column temperature: Maintain at 70°C for 2 minutes after injection, then program to increase the temperature at the rate of 5°C per minute to 240°C and to maintain 240°C for 5 minutes.

Injection port temperature: A constant temperature of about 220°C.

Detector temperature: A constant temperature of about 260°C.

Carrier gas: Helium.

Flow rate: 2.4 mL per minute.

Splitless.

◇Time span of measurement: For 41 minutes after the solvent peak.◇

System suitability—

◇Test for required detectability:◇ Place about 50 mg each of stearic acid for gas chromatography and palmitic acid for gas chromatography in a small conical flask fitted with a reflux condenser. Add 5.0 mL of boron trifluoride-methanol TS, mix, and proceed in the same manner as directed for the preparation of the sample solution, and use the solution so obtained as the solution for system suitability test. ◇To exactly 1 mL of the solution for system suitability test add heptane to make exactly 10 mL. To exactly 1 mL of this solution add heptane to make exactly 10 mL. Further, to exactly 1 mL of this solution add heptane to make exactly 10 mL. Confirm that the peak area of methyl stearate obtained with 1 μL of this solution is equivalent to 0.05 to 0.15% of that with 1 μL of the solution for system suitability test.◇

System performance: When the procedure is run with 1 μL of the solution for system suitability test under the above operating conditions, the relative retention time of methyl palmitate to methyl stearate is about 0.9, and the resolution between these peaks is not less than 5.0.

System repeatability: When the test is repeated 6 times with the solution for system suitability test under the above operating conditions, the relative standard deviation of the peak areas of methyl palmitate and methyl stearate are not more than 3.0%, respectively, and the relative standard deviation of the ratios of the peak area of methyl palmitate to methyl stearate is not more than 1.0%.

Assay Transfer about 0.5 g of Magnesium Stearate, accurately weighed, to a 250-mL flask, add 50 mL of a mixture of ethanol (99.5) and 1-butanol (1:1), 5 mL of ammonia solution (28), 3 mL of ammonium chloride buffer solution (pH 10), 30.0 mL of 0.1 mol/L disodium dihydrogen ethylenediamine tetraacetate VS and 1 to 2 drops of eriochrome black T TS, and mix. Heat at 45 – 50°C to make the solution clear, and after cooling, titrate <*2.50*> the excess disodium dihydrogen ethylenediamine tetraacetate with 0.1 mol/L zinc sulfate VS until the solution changes from blue to violet in color. Perform a blank determination in the same manner.

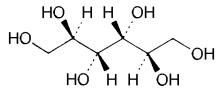
Each mL of 0.1 mol/L disodium dihydrogen ethylenediamine tetraacetate VS
= 2.431 mg of Mg

◆**Containers and storage** Containers—Tight containers.◆

Change the following as follows:

D-Mannitol

D-マンニトール



$C_6H_{14}O_6$: 182.17
D-Mannitol
[*69-65-8*]

This monograph is harmonized with the European Pharmacopoeia and the U.S. Pharmacopeia.

The corresponding part of the attributes/provisions which are agreed as non-harmonized within the scope of the harmonization is marked with symbols (◆ ◆), and the corresponding parts which are agreed as the JP local requirement other than the scope of the harmonization are marked with symbols (◇ ◇).

Information on the harmonization with the European Pharmacopoeia and the U.S. Pharmacopeia is available on the website of Pharmaceuticals and Medical Devices Agency.

D-Mannitol contains not less than 97.0% and not more than 102.0% of D-mannitol ($C_6H_{14}O_6$), calculated on the dried basis.

◆**Description** D-Mannitol occurs as white, crystals, powder or grain. It has a sweet taste with a cold sensation.

It is freely soluble in water, and practically insoluble in ethanol (99.5).

It dissolves in sodium hydroxide TS.

It shows crystal polymorphism.◆

Identification Determine the infrared absorption spectrum of D-Mannitol as directed in the potassium bromide disk method under Infrared Spectrophotometry ⟨*2.25*⟩, and compare the spectrum with the Reference Spectrum or the spectrum of D-Mannitol RS: both spectra exhibit similar intensities of absorption at the same wave numbers. If any difference appears between the spectra, put 25 mg each of D-Mannitol and D-Mannitol RS in glass vessels, dissolve in 0.25 mL of water without heating, dry them in a 600 – 700 W microwave oven for 20 minutes or in a drying chamber at 100°C for 1 hour, then further dry by gradual reducing pressure, and perform the same test as above with so obtained non-sticky white to pale yellow powders: both spectra exhibit similar intensities of absorption at the same wave numbers.

Melting point ⟨*2.60*⟩ 165 – 170°C

Purity **(1)** Clarity and color of solution—Dissolve 5.0 g of D-Mannitol in water to make 50 mL, and use this solution as the test solution. Perform the test with the test solution as directed under Turbidity Measurement ⟨*2.61*⟩: the solution is clear. Perform the test with the test solution according to Method 2 under Methods for Color Matching ⟨*2.65*⟩: the solution is colorless.

(2) Nickel—Shake 10.0 g of D-Mannitol with 30 mL of 2 mol/L acetic acid TS, and add water to make exactly 100 mL. Add 2.0 mL of a saturated solution of ammonium pyrrolidinedithiocarbamate (about 10 g/L) and 10.0 mL of water-saturated 4-methyl-2-pentanone, and shake for 30 seconds without exposure to light. Allow the layers to separate, and use the 4-methyl-2-pentanone layer as the sample solution. Separately, put 10.0 g each of D-Mannitol in three vessels, add 30 mL of 2 mol/L acetic acid TS to them, shake, add a suitable amount of water and exactly 0.5 mL, 1.0 mL and 1.5 mL respectively of Standard Nickel Solution for Atomic Absorption Spectrophotometry, and add water to make them exactly 100 mL. Then, proceed in the same manner as the sample solution, and use so obtained three 4-methyl-2-pentanone layers as the standard solutions. Additionally, prepare a 4-methyl-2-pentanone layer by proceeding in the same manner as the sample solution without using D-Mannitol, and use this layer as the blank solution. Perform the test with the sample solution and standard solutions as directed in the standard addition method under Atomic Absorption Spectrophotometry ⟨*2.23*⟩ according to the following conditions. Set the zero of the instrument using the blank solution, and between each measurement, rinse with water and ascertain that the readings return to zero with the blank solution: amount of nickel is not more than 1 ppm.

Gas: Combustible gas—Acetylene.
Supporting gas—Air.
Lamp: Nickel hollow-cathode lamp.
Wavelength: 232.0 nm.

(3) Related substances—Dissolve 0.50 g of D-Mannitol in water to make 10 mL, and use this solution as the sample solution. Pipet 2 mL of the sample solution, add water to make exactly 100 mL, and use this solution as the standard solution (1). Pipet 0.5 mL of the standard solution (1), add water to make exactly 20 mL, and use this solution as the standard solution (2). Perform the test with exactly 20 μL each of the sample solution and standard solutions (1) and (2) as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions. Determine each peak area by the automatic integration method: the peak area of D-sorbitol, having the relative retention time of about 1.2 to D-mannitol, obtained from the sample solution is not larger than that of D-mannitol from the standard solution (1) (not more than 2.0%), the total peak area of maltitol, having the relative retention time of about 0.69, and isomalt, having the relative retention times of about 0.6 and about 0.73, is not larger than the peak area of D-mannitol from the standard solution (1) (not more than 2.0%), and the area of the peak other than D-mannitol and the peaks mentioned above is not larger than 2 times the peak area of D-mannitol from the standard solution (2) (not more than 0.1%). Furthermore, the total area of the peak other than D-mannitol from the sample solution is not larger than the peak area of D-mannitol from the standard solution (1) (not more than 2.0%). For these calculations exclude the peak of which

area is not larger than the peak area of D-mannitol from the standard solution (2) (not more than 0.05%).

Operating conditions—

Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay.

Time span of measurement: About 1.5 times as long as the retention time of D-mannitol.

System suitability—

System performance: Proceed as directed in the system suitability in the Assay.

◇Test for required detectability: Confirm that the peak area of D-mannitol obtained with 20 μL of the standard solution (2) is equivalent to 1.75 to 3.25% of that with 20 μL of the standard solution (1).

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution (1) under the above operating conditions, the relative standard deviation of the peak area of D-mannitol is not more than 1.0%.◇

(4) Glucose—To 7.0 g of D-Mannitol add 13 mL of water and 40 mL of Fehling's TS, boil gently for 3 minutes, and allow to stand for 2 minutes to precipitate copper (I) oxide. Separate the supernatant liquid, filter through a sintered glass filter for cupric oxide filtration coated with siliceous earth or a sintered glass filter (G4). Wash the precipitates with 50 – 60°C hot water until the washing no longer alkaline, and filter the washings through the filter described above. Discard all the filtrate at this step. Immediately, dissolve the precipitate with 20 mL of iron (III) sulfate TS, filter through the filter described above in a clean flask, and wash the filter with 15 – 20 mL of water. Combine the filtrate and the washings, heat to 80°C, and titrate <2.50> with 0.02 mol/L potassium permanganate VS until the green color turns to light red and the color persists at least 10 seconds: not more than 3.2 mL is required to change the color of the solution (not more than 0.1% expressed as glucose).

Conductivity <2.51> Dissolve 20.0 g of D-Mannitol in a fleshly boiled and cooled water prepared from distilled water by heating to 40 – 50°C, add the same water to make 100 mL, and use this solution as the sample solution. After cooling, measure the conductivity of the sample solution at 25 ± 0.1°C while gently stirring with a magnetic stirrer: not more than 20 $\mu S \cdot cm^{-1}$.

Loss on drying <2.41> Not more than 0.5% (1 g, 105°C, 4 hours).

Assay Weigh accurately about 0.5 g each of D-Mannitol and D-Mannitol RS (separately determine the loss on drying <2.41> under the same conditions as D-Mannitol), dissolve separately in water to make exactly 10 mL, and use these solutions as the sample solution and the standard solution, respectively. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the peak areas, A_T and A_S, of D-mannitol in each solution.

$$\text{Amount (g) of D-mannitol } (C_6H_{14}O_6) = M_S \times A_T/A_S$$

M_S: Amount (g) of D-Mannitol RS taken, calculated on the dried basis

Operating conditions—

Detector: A differential refractometer maintained at a constant temperature (40°C for example).

Column: A stainless steel column 7.8 mm in inside diameter and 30 cm in length, packed with strongly acidic ion-exchange resin for liquid chromatography (calcium type) composed with a sulfonated polystyrene cross-linked with 8% of divinylbenzene (9 μm in particle diameter).

Column temperature: 85 ± 2°C.

Mobile phase: Water.

Flow rate: 0.5 mL per minute (the retention time of D-mannitol is about 20 minutes).

System suitability—

System performance: Dissolve 0.25 g each of D-Mannitol and D-sorbitol in water to make 10 mL, and use this solution as the solution for system suitability test (1). Separately, dissolve 0.5 g each of maltitol and isomalt in water to make 100 mL. To 2 mL of this solution add water to make 10 mL, and use this solution as the solution for system suitability test (2). When proceed with 20 μL each of the solution for system suitability test (1) and the solution for system suitability test (2) as directed under the above operating conditions, isomalt (first peak), maltitol, isomalt (second peak), D-mannitol and D-sorbitol are eluted in this order, the relative retention time of isomalt (first peak), maltitol, isomalt (second peak) and D-sorbitol to D-mannitol is about 0.6, about 0.69, about 0.73 and about 1.2, respectively, and the resolution between the peaks of D-mannitol and D-sorbitol is not less than 2.0. Coelution of maltitol and the second peak of isomalt may be observed.

◇System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of D-mannitol is not more than 1.0%.◇

◆**Containers and storage** Containers—Well-closed containers.◆

dl-Menthol

dl-メントール

Change the Containers and storage as follows:

Containers and storage Containers—Tight containers.

l-Menthol

l-メントール

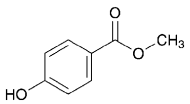
Change the Containers and storage as follows:

Containers and storage Containers—Tight containers.

Change the following as follows:

Methyl Parahydroxybenzoate

パラオキシ安息香酸メチル



$C_8H_8O_3$: 152.15
Methyl 4-hydroxybenzoate
[*99-76-3*]

This monograph is harmonized with the European Pharmacopoeia and the U.S. Pharmacopeia.

The corresponding part of the attributes/provisions which are agreed as non-harmonized within the scope of the harmonization is marked with symbols (◆ ◆), and the corresponding parts which are agreed as the JP local requirement other than the scope of the harmonization are marked with symbols (◇ ◇).

Information on the harmonization with the European Pharmacopoeia and the U.S. Pharmacopeia is available on the website of the Pharmaceuticals and Medical Devices Agency.

Methyl Parahydroxybenzoate contains not less than 98.0% and not more than 102.0% of methyl parahydroxybenzoate ($C_8H_8O_3$).

◆**Description** Methyl Parahydroxybenzoate, occurs as colorless crystals or a white crystalline powder.

It is freely soluble in methanol, in ethanol (95) and in acetone, and slightly soluble in water.◆

Identification Determine the infrared absorption spectrum of Methyl Parahydroxybenzoate as directed in the potassium bromide disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum or the spectrum of Methyl Parahydroxybenzoate RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

Melting point <*2.60*> 125 – 128°C

Purity **(1)** Clarity and color of solution—Dissolve 1.0 g of Methyl Parahydroxybenzoate in ethanol (95) to make 10 mL: the solution is clear and not more intensely colored than ethanol (95) or the following control solution.

Control solution: To 5.0 mL of Cobalt (II) Chloride CS, 12.0 mL of Iron (III) Chloride CS and 2.0 mL of Copper (II) Sulfate CS add diluted dilute hydrochloric acid (1 in 10) to make 1000 mL.

(2) Acidity—To 2 mL of the solution of Methyl Parahydroxybenzoate obtained in (1) add 3 mL of ethanol (95), add 5 mL of freshly boiled and cooled water and 0.1 mL of bromocresol green-sodium hydroxide-ethanol TS, then add 0.1 mol/L sodium hydroxide VS until the solution shows a blue color: the volume of 0.1 mol/L sodium hydroxide VS used does not exceed 0.1 mL.

(3) Related substances—Dissolve 50.0 mg of Methyl Parahydroxybenzoate in 2.5 mL of methanol, and add the mobile phase to make exactly 50 mL. Pipet 10 mL of this solution, add the mobile phase to make exactly 100 mL, and use this solution as the sample solution. Pipet 1 mL of the sample solution, and add the mobile phase to make exactly 20 mL. Pipet 1 mL of this solution, add the mobile phase to make exactly 10 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine each peak area by the automatic integration method: the peak area of parahydroxybenzoic acid having the relative retention time of about 0.6 to methyl parahydroxybenzoate obtained from the sample solution is not larger than the peak area of methyl parahydroxybenzoate from the standard solution (0.5%). For the peak area of parahydroxybenzoic acid, multiply the correction factor, 1.4. Furthermore, the area of the peak other than methyl parahydroxybenzoate and parahydroxybenzoic acid from the sample solution is not larger than the peak area of methyl parahydroxybenzoate from the standard solution (0.5%), and the total area of the peaks other than methyl parahydroxybenzoate from the sample solution is not larger than 2 times the peak area of methyl parahydroxybenzoate from the standard solution (1.0%). For this calculation the peak area not larger than 1/5 times the peak area of methyl parahydroxybenzoate from the standard solution is excluded (0.1%).

Operating conditions—

Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay.

Time span of measurement: 5 times as long as the retention time of methyl parahydroxybenzoate.

System suitability—

System performance: Proceed as directed in the system suitability in the Assay.

◇Test for required detectability: To exactly 2 mL of the standard solution add the mobile phase to make exactly 10 mL. Confirm that the peak area of methyl parahydroxybenzoate obtained with 10 μL of this solution is equivalent to 14 to 26% of that with 10 μL of the standard solution.◇

◇System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of methyl parahydroxybenzoate is not more than 2.0%.◇

Residue on ignition ⟨*2.44*⟩ Not more than 0.1% (1 g).

Assay Weigh accurately about 50 mg each of Methyl Parahydroxybenzoate and Methyl Parahydroxybenzoate RS, dissolve separately in 2.5 mL each of methanol, and add the mobile phase to make exactly 50 mL. Pipet 10 mL each of these solutions, add the mobile phase to make exactly 100 mL, and use these solutions as the sample solution and the standard solution, respectively. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, and determine the peak areas, A_T and A_S, of methyl parahydroxybenzoate in each solution.

Amount (mg) of methyl parahydroxybenzoate ($C_8H_8O_3$) $= M_S \times A_T/A_S$

M_S: Amount (mg) of Methyl Parahydroxybenzoate RS taken

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 272 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

◇Column temperature: A constant temperature of about 35°C.◇

Mobile phase: A mixture of methanol and potassium dihydrogen phosphate solution (17 in 2500) (13:7).

Flow rate: 1.3 mL per minute.

System suitability—

System performance: Dissolve 5 mg each of Methyl Parahydroxybenzoate and parahydroxybenzoic acid in the mobile phase to make exactly 100 mL. Pipet 1 mL of this solution, and add the mobile phase to make exactly 10 mL. When the procedure is run with 10 μL of this solution under the above operating conditions, parahydroxybenzoic acid and methyl parahydroxybenzoate are eluted in this order, the relative retention time of parahydroxybenzoic acid to methyl parahydroxybenzoate is about 0.6, and the resolution between these peaks is not less than 2.0.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of methyl parahydroxybenzoate is not more than 0.85%.

◆**Containers and storage** Containers—Well-closed containers.◆

Delete the following Monographs:

Nartograstim (Genetical Recombination)

ナルトグラスチム(遺伝子組換え)

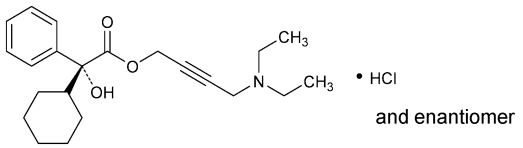
Nartograstim for Injection (Genetical Recombination)

注射用ナルトグラスチム(遺伝子組換え)

Add the following:

Oxybutynin Hydrochloride

オキシブチニン塩酸塩



$C_{22}H_{31}NO_3$.HCl: 393.95

4-(Diethylamino)but-2-yn-1-yl (2*RS*)-2-cyclohexyl-2-hydroxy-2-phenylacetate monohydrochloride

[*1508-65-2*]

Oxybutynin Hydrochloride, when dried, contains not less than 98.0% and not more than 101.0% of oxybutynin hydrochloride ($C_{22}H_{31}NO_3$.HCl).

Description Oxybutynin Hydrochloride occurs as a white crystalline powder.

It is freely soluble in water and in ethanol (99.5).

A solution of Oxybutynin Hydrochloride (1 in 50) shows no optical rotation.

Identification **(1)** Determine the absorption spectrum of a solution of Oxybutynin Hydrochloride (3 in 100,000) as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Oxybutynin Hydrochloride, previously dried, as directed in the potassium chloride disk method under Infrared Spectrophotometry ⟨*2.25*⟩, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) A solution of Oxybutynin Hydrochloride (1 in 50) responds to Qualitative Tests ⟨*1.09*⟩ for chloride.

Melting point ⟨*2.60*⟩ 124 – 129°C

Purity Related substances—Dissolve 50 mg of Oxybutynin Hydrochloride in 10 mL of the mobile phase, and use this

solution as the sample solution. Pipet 1 mL of the sample solution, add the mobile phase to make exactly 200 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine each peak area by the automatic integration method: the peak area of the related substance A, having the relative retention time of about 1.6 to oxybutynin, obtained from the sample solution is not larger than 3 times the peak area of oxybutynin from the standard solution, and the area of the peak other than oxybutynin and the peak mentioned above from the sample solution is not larger than 1/5 times the peak area of oxybutynin from the standard solution. Furthermore, the total area of the peaks other than oxybutynin and the related substance A from the sample solution is not larger than the peak area of oxybutynin from the standard solution. For the peak area of the related substance A, multiply the correction factor, 2.3.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 210 nm).

Column: A stainless steel column 3.9 mm in inside diameter and 15 cm in length, packed with octylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: Dissolve 3.4 g of potassium dihydrogen phosphate and 4.36 g of dipotassium hydrogen phosphate in water to make 1000 mL. To 490 mL of this solution add 510 mL of acetonitrile for liquid chromatography.

Flow rate: Adjust so that the retention time of oxybutynin is about 15 minutes.

Time span of measurement: About 2 times as long as the retention time of oxybutynin.

System suitability—

Test for required detectability: Pipet 2 mL of the standard solution, and add the mobile phase to make exactly 20 mL. Confirm that the peak area of oxybutynin obtained with 10 μL of this solution is equivalent to 7 to 13% of that with 10 μL of the standard solution.

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of oxybutynin are not less than 5000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of oxybutynin is not more than 2.0%.

Loss on drying <2.41> Not more than 3.0% (0.5 g, 105°C, 4 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

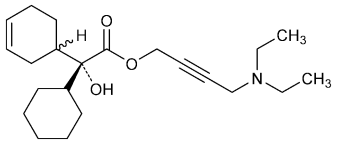
Assay Weigh accurately about 0.5 g of Oxybutynin Hydrochloride, previously dried, dissolve in 70 mL of a mixture of acetic anhydride and acetic acid (100) (7:3), and titrate <2.50> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 39.40 mg of $C_{22}H_{31}NO_3.HCl$

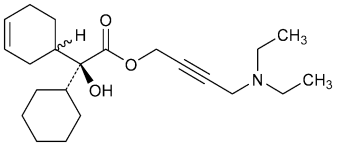
Containers and storage Containers—Tight containers.
Storage—Light-resistant.

Others

Related substance A:
4-(Diethylamino)but-2-yn-1-yl (2*R*)-2-(cyclohex-3-en-1-yl)-2-cyclohexyl-2-hydroxyacetate



4-(Diethylamino)but-2-yn-1-yl (2*S*)-2-(cyclohex-3-en-1-yl)-2-cyclohexyl-2-hydroxyacetate



Change the following as follows:

White Petrolatum

白色ワセリン

This monograph is harmonized with the European Pharmacopoeia and the U.S. Pharmacopeia.

The corresponding part of the attributes/provisions which are agreed as non-harmonized within the scope of the harmonization is marked with symbol (◆ ◆), and the corresponding parts which are agreed as the JP local requirement other than the scope of the harmonization are marked with symbols (◇ ◇).

Information on the harmonization with the European Pharmacopoeia and the U.S. Pharmacopeia is available on the website of the Pharmaceuticals and Medical Devices Agency.

White Petrolatum is a purified and wholly or nearly decolorized semi-solid mixture of hydrocarbons obtained from petroleum.

It may contain a suitable antioxidant ◇such as dibutylhydroxytoluene or an appropriate type of tocopherol.◇ ◆If an antioxidant is added, the label states the name and amount of the antioxidant.◆

◆**Description** White Petrolatum occurs as a white to pale yellow, homogeneous, unctuous mass. It is odorless and tasteless.

It is practically insoluble in water and in ethanol (95).

It becomes a clear liquid when warmed.◆

Identification Place about 2 mg of White Petrolatum on an optical plate, place the plate upon another optical plate to spread the sample, and determine the infrared absorption spectrum as directed in the liquid film method under Infrared Spectrophotometry ⟨*2.25*⟩, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

◇**Melting point** ⟨*2.60*⟩ 38 – 60°C (Method 3).◇

Purity **(1)** Color—Melt about 10 g of White Petrolatum on a water bath, and pour 5 mL of it into a clear glass 15 × 150 mm test tube, and keep the content in melted condition: the liquid has no more color than the following control solution, when observed transversely in reflected light against a white background.

Control solution: Pipet 0.5 mL of Iron (III) Chloride CS and 4.5 mL of diluted dilute hydrochloric acid (1 in 10), respectively, and mix in a clear glass 15 × 150 mm test tube.

(2) Acidity or alkalinity—To 10 g of White Petrolatum add 20 mL of hot water, shake vigorously for 1 minute, and cool. To the 10 mL of aqueous layer add 0.1 mL of phenolphthalein TS: the solution is colorless. Add 0.01 mol/L sodium hydroxide VS until a light red or red color develops: the necessary volume of the VS is not more than 0.5 mL.

(3) Polycyclic aromatic hydrocarbons—Dissolve 1.0 g of White Petrolatum in the solution prepared by adding 50 mL of hexane for ultraviolet-visible spectrophotometry to 10 mL of dimethylsulfoxide for ultraviolet-visible spectrophotometry and shaking twice. Transfer this solution to a separator with unlubricated ground-glass parts (stopper, stopcock). To this separator add 20 mL of dimethylsulfoxide for ultraviolet-visible spectrophotometry, shake vigorously for 1 minute, and allow to stand until two clear layers are formed. Transfer the lower layer to a second separator, repeat the extraction with a further 20 mL of dimethylsulfoxide for ultraviolet-visible spectrophotometry. Combine the lower layers obtained by the extraction procedures, shake vigorously with 20 mL of hexane for ultraviolet-visible spectrophotometry for 1 minute. Allow to stand until two clear layers are formed, separate the lower layer, add dimethylsulfoxide for ultraviolet-visible spectrophotometry to make exactly 50 mL, and use this solution as the sample solution. Determine the absorbance between 265 nm and 420 nm in a layer of 1 cm. Shake vigorously 25 mL of hexane for ultraviolet-visible spectrophotometry and 10 mL of dimethylsulfoxide for ultraviolet-visible spectrophotometry for 1 minute, allow to stand until two clear layers are formed, and use the lower layer thus obtained as the control solution. Separately, weigh accurately about 6 mg of naphthalene, dissolve in dimethylsulfoxide for ultraviolet-visible spectrophotometry to make exactly 100 mL. Pipet 10 mL of this solution, add dimethylsulfoxide for ultraviolet-visible spectrophotometry to make exactly 100 mL, and use this solution as the standard solution. Determine the absorbance of the standard solution at 278 nm in a layer of 1 cm in length and the absorption spectrum between 265 nm and 420 nm of the sample solution as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩: the maximum absorbance of the sample solution is not more than 1/4 the absorbance at 278 nm of the standard solution.

Residue on ignition ⟨*2.44*⟩ Not more than 0.05% (2 g).

◆**Containers and storage** Containers—Tight containers.◆

Change the following as follows:

Yellow Petrolatum

Petrolatum

黄色ワセリン

This monograph is harmonized with the European Pharmacopoeia and the U.S. Pharmacopeia.

The corresponding part of the attributes/provisions which are agreed as non-harmonized within the scope of the harmonization is marked with symbol (◆ ◆), and the corresponding parts which are agreed as the JP local requirement other than the scope of the harmonization are marked with symbols (◇ ◇).

Information on the harmonization with the European Pharmacopoeia and the U.S. Pharmacopeia is available on the website of the Pharmaceuticals and Medical Devices Agency.

Yellow Petrolatum is a purified semi-solid mixture of hydrocarbons obtained from petroleum.

It may contain a suitable antioxidant ◇such as dibutylhydroxytoluene or an appropriate type of tocopherol.◇ ◆If an antioxidant is added, the label states the name and amount of the antioxidant.◆

◆**Description** Yellow Petrolatum occurs as a yellow, homogeneous, unctuous mass. It is odorless and tasteless.

It is slightly soluble in ethanol (95), and practically insoluble in water.

It becomes a yellow, clear liquid with slight fluorescence when warmed.◆

Identification Place about 2 mg of Yellow Petrolatum on an optical plate, place the plate upon another optical plate to spread the sample, and determine the infrared absorption spectrum as directed in the liquid film method under Infrared Spectrophotometry ⟨*2.25*⟩, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

◇**Melting point** ⟨*2.60*⟩ 38 – 60°C (Method 3).◇

Purity **(1)** Color—Melt about 10 g of Yellow Petrolatum on a water bath, and pour 5 mL of it into a clear glass 15 × 150 mm test tube, and keep the content in melted condition: the liquid has no more color than the following control solution (1) and has equal color to or more color than the following control solution (2), when observed transversely in

reflected light against a white background.

Control solution (1): Pipet 3.8 mL of Iron (III) Chloride CS and 1.2 mL of Cobalt (II) Chloride CS, and mix in a clear glass 15 × 150 mm test tube.

Control solution (2): Pipet 0.5 mL of Iron (III) Chloride CS and 4.5 mL of diluted dilute hydrochloric acid (1 in 10), and mix in a clear glass 15 × 150 mm test tube.

(2) Acidity or alkalinity—To 10 g of Yellow Petrolatum add 20 mL of hot water, shake vigorously for 1 minute, and cool. To the 10 mL of aqueous layer add 0.1 mL of phenolphthalein TS: the solution is colorless. Add 0.01 mol/L sodium hydroxide VS until a light red or red color develops: the necessary volume of the VS is not more than 0.5 mL.

(3) Polycyclic aromatic hydrocarbons—Dissolve 1.0 g of Yellow Petrolatum in the solution prepared by adding 50 mL of hexane for ultraviolet-visible spectrophotometry to 10 mL of dimethylsulfoxide for ultraviolet-visible spectrophotometry and shaking twice. Transfer this solution to a separator with unlubricated ground-glass parts (stopper, stopcock). To this separator add 20 mL of dimethylsulfoxide for ultraviolet-visible spectrophotometry, shake vigorously for 1 minute, and allow to stand until two clear layers are formed. Transfer the lower layer to a second separator, repeat the extraction with a further 20 mL of dimethylsulfoxide for ultraviolet-visible spectrophotometry. Combine the lower layers obtained by the extraction procedures, shake vigorously with 20 mL of hexane for ultraviolet-visible spectrophotometry for 1 minute. Allow to stand until two clear layers are formed, separate the lower layer, add dimethylsulfoxide for ultraviolet-visible spectrophotometry to make exactly 50 mL, and use this solution as the sample solution. Determine the absorbance between 265 nm and 420 nm in a layer of 1 cm. Shake vigorously 25 mL of hexane for ultraviolet-visible spectrophotometry and 10 mL of dimethylsulfoxide for ultraviolet-visible spectrophotometry for 1 minute, allow to stand until two clear layers are formed, and use the lower layer thus obtained as the control solution. Separately, weigh accurately about 6 mg of naphthalene, dissolve in dimethylsulfoxide for ultraviolet-visible spectrophotometry to make exactly 100 mL. Pipet 10 mL of this solution, add dimethylsulfoxide for ultraviolet-visible spectrophotometry to make exactly 100 mL, and use this solution as the standard solution. Determine the absorbance of the standard solution at 278 nm in a layer of 1 cm in length and the absorption spectrum between 265 nm and 420 nm of the sample solution as directed under Ultraviolet-visible Spectrophotometry <*2.24*>: the maximum absorbance of the sample solution is not more than 1/4 the absorbance at 278 nm of the standard solution.

Residue on ignition <*2.44*> Not more than 0.05% (2 g).

◆**Containers and storage** Containers—Tight containers.◆

Change the following as follows:

Polysorbate 80

ポリソルベート**80**

This monograph is harmonized with the European Pharmacopoeia and the U.S. Pharmacopeia.

The corresponding part of the attributes/provisions which are agreed as non-harmonized within the scope of the harmonization is marked with symbols (◆ ◆), and the corresponding parts which are agreed as the JP local requirement other than the scope of the harmonization are marked with symbols (◇ ◇).

Information on the harmonization with the European Pharmacopoeia and the U.S. Pharmacopeia is available on the website of the Pharmaceuticals and Medical Devices Agency.

Polysorbate 80 is a mixture of partial esters of fatty acids, mainly oleic acid, with sorbitol and its anhydrides ethoxylated with approximately 20 moles of ethylene oxide for each mole of sorbitol and sorbitol anhydrides.

◆**Description** Polysorbate 80 is a colorless or brownish yellow, clear or slightly opalescent, oily liquid.

It is miscible with water, with methanol, with ethanol (99.5) and with ethyl acetate.

It is practically insoluble in fatty oils and in liquid paraffin.

Viscosity: about 400 mPa·s (25°C).

Specific gravity d_{20}^{20} : about 1.10◆

Identification It meets the requirements of the Composition of fatty acids.

Composition of fatty acids Dissolve 0.10 g of Polysorbate 80 in 2 mL of a solution of sodium hydroxide in methanol (1 in 50) in a 25-mL conical flask, and boil under a reflux condenser for 30 minutes. Add 2.0 mL of boron trifluoride-methanol TS through the condenser, and boil for 30 minutes. Add 4 mL of heptane through the condenser, and boil for 5 minutes. After cooling, add 10.0 mL of saturated sodium chloride solution, shake for about 15 seconds, and add a quantity of saturated sodium chloride solution such that the upper layer is brought into the neck of the flask. Collect 2 mL of the upper layer, wash with three 2-mL portions of water, dry with anhydrous sodium sulfate, and use this solution as the sample solution. Perform the test with 1 μL each of the sample solution and fatty acid methyl esters mixture TS as directed under Gas Chromatography <*2.02*> according to the following conditions. Identify each peak obtained with the sample solution using the chromatogram with fatty acid methyl esters mixture TS. Determine each peak area with the sample solution by the automatic integration method, and calculate the composition of fatty acids by the area percentage method: myristic acid is not more than 5.0%, palmitic acid is not more than 16.0%, palmitoleic acid is not more than 8.0%, stearic acid is not

more than 6.0%, oleic acid is not less than 58.0%, linoleic acid is not more than 18.0% and linolenic acid is not more than 4.0%.

Operating conditions—

Detector: A hydrogen flame-ionization detector.

Column: A fused silica column 0.32 mm in inside diameter and 30 m in length, coated with polyethylene glycol 20 M for gas chromatography 0.5 μm in thickness.

Column temperature: Inject at a constant temperature of about 80°C, raise the temperature to 220°C at a rate of 10°C per minute, and maintain at 220°C for 40 minutes.

Injection port temperature: A constant temperature of about 250°C.

Detector temperature: A constant temperature of about 250°C.

Carrier gas: Helium.

Flow rate: 50 cm per second.

Split ratio: 1: 50.

System suitability—

Test for required detectability: Dissolve 0.50 g of the mixture of fatty acid methyl esters described in the following table in heptane to make exactly 50 mL, and use this solution as the solution for system suitability test. Pipet 1 mL of the solution for system suitability test add heptane to make exactly 10 mL. When the procedure is run with 1 μL of this solution under the above operating conditions, the SN ratio of methyl myristate is not less than 5.

Mixture of fatty acid methyl esters	Composition (%)
Methyl myristate for gas chromatography	5
Methyl palmitate for gas chromatography	10
Methyl stearate for gas chromatography	15
Methyl arachidate for gas chromatography	20
Methyl oleate for gas chromatography	20
Methyl eicosenoate for gas chromatography	10
Methyl behenate	10
Methyl lignocerate for gas chromatography	10

System performance: When the procedure is run with 1 μL of the solution for system suitability test under the above operating conditions, ◇methyl stearate and methyl oleate are eluted in this order,◇ the resolution between these peaks is not less than 1.8, and the number of theoretical plates of the peak of methyl stearate is not less than 30,000.

Acid value ⟨*1.13*⟩ Not more than 2.0 (using ◆ethanol (95)◆ instead).

Saponification value Introduce about 4 g of Polysorbate 80, accurately weighed, into a 250-mL borosilicate glass flask. Add exactly 30 mL of 0.5 mol/L potassium hydroxide-ethanol VS and a few glass beads. Attach a reflux condenser, and heat for 60 minutes. Add 1 mL of phenolphthalein TS and 50 mL of ethanol (99.5), and titrate ⟨*2.50*⟩ immediately with 0.5 mol/L hydrochloric acid VS. Perform a blank determination in the same manner. Calculate the saponification value by the following equation: 45 – 55.

$$\text{Saponification value} = (a - b) \times 28.05/M$$

M: Amount (g) of Polysorbate 80 taken

a: Volume (mL) of 0.5 mol/L hydrochloric acid VS required for blank determination

b: Volume (mL) of 0.5 mol/L hydrochloric acid VS required for sample determination

Hydroxyl value Introduce about 2 g of Polysorbate 80, accurately weighed, into a 150-mL round bottom flask, add exactly 5 mL of acetic anhydride-pyridine TS, and attach an air condenser. Heat the flask in a water bath for 1 hour keeping the level of the water about 2.5 cm above the level of the liquid in the flask. Withdraw the flask and allow to cool. Add 5 mL of water through the condenser. If a cloudiness appears add sufficient pyridine to clear it, noting the volume added. Shake the flask, and heat in the water bath for 10 minutes. Withdraw the flask and allow to cool. Rinse the condenser and the walls of the flask with 5 mL of neutralized ethanol, and titrate ⟨*2.50*⟩ with 0.5 mol/L potassium hydroxide-ethanol VS (indicator: 0.2 mL of phenolphthalein TS). Perform a blank determination in the same manner. Calculate the hydroxyl value by the following equation: 65 – 80.

$$\text{Hydroxyl value} = (a - b) \times 28.05/M + \text{acid value}$$

M: Amount (g) of Polysorbate 80 taken

a: Volume (mL) of 0.5 mol/L potassium hydroxide-ethanol VS required for blank determination

b: Volume (mL) of 0.5 mol/L potassium hydroxide-ethanol VS required for sample determination

Purity **(1)** Ethylene oxide and 1,4-dioxane—Transfer exactly 1.00 g of Polysorbate 80 into a 10-mL headspace vial, add exactly 2 mL of water, seal the vial immediately with a septum of silicon rubber coated with fluororesin and an aluminum cap. Mix carefully, and use the content as the sample solution. Separately, pipet 0.5 mL of a solution, prepared by dissolving ethylene oxide in dichloromethane so that each mL contains 50 mg, and add water to make exactly 50 mL. Allow to stand to reach room temperature. Pipet 1 mL of this solution, add water to make exactly 250 mL, and use this solution as ethylene oxide stock solution. Separately, pipet 1 mL of 1,4-dioxane, add water to make exactly 200 mL. Pipet 1 mL of this solution, add water to make exactly 100 mL, and use this solution as 1,4-dioxane stock solution. To exact 6 mL of ethylene oxide stock solution and exact 2.5 mL of 1,4-dioxane stock solution add water to make exactly 25 mL, and use this solution as ethylene oxide-1,4-dioxane standard stock solution. Separately, transfer exactly 1.00 g of Polysorbate 80 into a 10-mL headspace vial, add exactly 2 mL of ethylene oxide-1,4-dioxane standard stock solution, seal the vial immediately with a septum of silicon rubber coated the surface with fluororesin and an aluminum cap. Mix carefully, and use the content as the standard solution. Perform the test with the sample solution and standard solution as directed in the head-space method under Gas Chromatography ⟨*2.02*⟩ according to the following conditions. The amounts of ethylene oxide and

1,4-dioxane, calculated by the following equations, are not more than 1 ppm and not more than 10 ppm, respectively.

$$\text{Amount (ppm) of ethylene oxide} = 2 \times C_{EO} \times A_a/(A_b - A_a)$$

C_{EO}: Concentration (μg/mL) of added ethylene oxide in the standard solution
A_a: Peak area of ethylene oxide obtained with the sample solution
A_b: Peak area of ethylene oxide obtained with the standard solution

$$\text{Amount (ppm) of 1,4-dioxane} = 2 \times 1.03 \times C_D \times A'_a \times 1000/(A'_b - A'_a)$$

C_D: Concentration (μL/mL) of added 1,4-dioxane in the standard solution
1.03: Density (g/mL) of 1,4-dioxane
A'_a: Peak area of 1,4-dioxane obtained with the sample solution
A'_b: Peak area of 1,4-dioxane obtained with the standard solution

Head-space injection conditions—
Equilibration temperature in vial: A constant temperature of about 80°C.
Equilibration time in vial: 30 minutes.
Carrier gas: Helium.
Injection volume of sample: 1.0 mL.
Operating conditions—
Detector: A hydrogen flame-ionization detector.
Column: A fused silica column 0.53 mm in inside diameter and 50 m in length, coated the inside surface with 5% diphenyl-95% dimethylpolysiloxane for gas chromatography 5 μm in thickness.
Column temperature: Inject at a constant temperature of about 70°C, raise the temperature to 250°C at a rate of 10°C per minute, and maintain at 250°C for 5 minutes.
Injection port temperature: A constant temperature of about 85°C.
Detector temperature: A constant temperature of about 250°C.
Carrier gas: Helium.
Flow rate: 4.0 mL per minute.
Split ratio: 1:3.5.
System suitability—
System performance: Introduce 0.100 g of acetaldehyde in a 100-mL volumetric flask, and add water to make 100 mL. To exact 1 mL of this solution add water to make exactly 100 mL. Transfer exactly 2 mL of this solution and exactly 2 mL of ethylene oxide stock solution into a 10-mL headspace vial, seal the vial immediately with a fluororesin coated silicon septum and an aluminum cap. Mix carefully, and use the content as the solution for system suitability test. When perform the test with the standard solution and the solution for system suitability test under the above conditions, acetaldehyde, ethylene oxide and 1,4-dioxane are eluted in this order, and the resolution between the peaks of acetaldehyde and ethylene oxide is not less than 2.0.

(2) Peroxide value—Introduce about 10 g of Polysorbate 80, accurately weighed, into a 100-mL beaker, dissolve in 20 mL of acetic acid (100). Add 1 mL of saturated potassium iodide solution and allow to stand for 1 minute. Add 50 mL of fleshly boiled and cooled water, and titrate <2.50> with 0.01 mol/L sodium thiosulfate VS, while stirring with a magnetic stirrer (potentiometric titration). Perform a blank determination in the same manner, and make any necessary correction. Calculate peroxide value by the following equation: not more than 10.0.

$$\text{Peroxide value} = (a - b) \times 10/M$$

M: Amount (g) of Polysorbate 80 taken
a: Volume (mL) of 0.01 mol/L sodium thiosulfate VS required for sample determination
b: Volume (mL) of 0.01 mol/L sodium thiosulfate VS required for blank determination

Water <2.48> Not more than 3.0% (1 g, volumetric titration, direct titration).

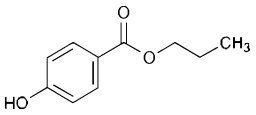
Residue on ignition Heat a quartz or platinum crucible to redness for 30 minutes, allow to cool in a desiccator (silica gel or other appropriate desiccants), and weigh accurately. Evenly distribute 2.00 g of Polysorbate 80 in the crucible, dry at 100 – 105°C for 1 hour, ◇and gradually heat with as lower temperature as possible to carbonize completely.◇ Then after igniting to constant mass in an electric furnace at 600 ± 25°C, allow the crucible to cool in a desiccator, and weigh the mass accurately. Flames should not be produced at any time during the procedure. If after prolonged ignition the ash still contains black particles, take up the ash with hot water, filter through a filter paper for quantitative analysis, and ignite the residue and the filter paper. Combine the filtrate with the ash, carefully evaporate to dryness, and ignite to constant mass: not more than 0.25%.

Containers and storage Containers—Tight containers.
Storage—Light-resistant.

Change the following as follows:

Propyl Parahydroxybenzoate

パラオキシ安息香酸プロピル



$C_{10}H_{12}O_3$: 180.20
Propyl 4-hydroxybenzoate
[*94-13-3*]

This monograph is harmonized with the European Pharmacopoeia and the U.S. Pharmacopeia.

The corresponding part of the attributes/provisions which are agreed as non-harmonized within the scope of the harmonization is marked with symbols (◆ ◆), and the cor-

responding parts which are agreed as the JP local requirement other than the scope of the harmonization are marked with symbols (◇ ◇).

Information on the harmonization with the European Pharmacopoeia and the U.S. Pharmacopeia is available on the website of the Pharmaceuticals and Medical Devices Agency.

Propyl Parahydroxybenzoate

Propyl Parahydroxybenzoate contains not less than 98.0% and not more than 102.0% of propyl parahydroxybenzoate ($C_{10}H_{12}O_3$).

◆**Description** Propyl Parahydroxybenzoate occurs as colorless crystals or a white crystalline powder.

It is freely soluble in methanol, in ethanol (95) and in acetone, and very slightly soluble in water.◆

Identification Determine the infrared absorption spectrum of Propyl Parahydroxybenzoate as directed in the potassium bromide disk method under Infrared Spectrophotometry ⟨*2.25*⟩, and compare the spectrum with the Reference Spectrum or the spectrum of Propyl Parahydroxybenzoate RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

Melting point ⟨*2.60*⟩ 96 – 99°C

Purity **(1)** Clarity and color of solution—Dissolve 1.0 g of Propyl Parahydroxybenzoate in ethanol (95) to make 10 mL: the solution is clear and not more intensely colored than ethanol (95) or the following control solution.

Control solution: To 5.0 mL of Cobalt (II) Chloride CS, 12.0 mL of Iron (III) Chloride CS and 2.0 mL of Copper (II) Sulfate CS add diluted dilute hydrochloric acid (1 in 10) to make 1000 mL.

(2) Acidity—To 2 mL of the solution of Propyl Parahydroxybenzoate obtained in (1) add 3 mL of ethanol (95), add 5 mL of freshly boiled and cooled water and 0.1 mL of bromocresol green-sodium hydroxide-ethanol TS, then add 0.1 mol/L sodium hydroxide VS until the solution shows a blue color: the volume of 0.1 mol/L sodium hydroxide VS used does not exceed 0.1 mL.

(3) Related substances—Dissolve 50.0 mg of Propyl Parahydroxybenzoate in 2.5 mL of methanol, and add the mobile phase to make exactly 50 mL. Pipet 10 mL of this solution, add the mobile phase to make exactly 100 mL, and use this solution as the sample solution. Pipet 1 mL of the sample solution, and add the mobile phase to make exactly 20 mL. Pipet 1 mL of this solution, add the mobile phase to make exactly 10 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, and determine each peak area by the automatic integration method: the peak area of parahydroxybenzoic acid having the relative retention time of about 0.3 to propyl parahydroxybenzoate obtained from the sample solution is not larger than the peak area of propyl parahydroxybenzoate from the standard solution (0.5%). For the peak area of parahydroxybenzoic acid, multiply the correction factor, 1.4. Furthermore, the area of the peak other than propyl parahydroxybenzoate and parahydroxybenzoic acid from the sample solution is not larger than the peak area of propyl parahydroxybenzoate from the standard solution (0.5%), and the total area of the peaks other than propyl parahydroxybenzoate from the sample solution is not larger than 2 times the peak area of propyl parahydroxybenzoate from the standard solution (1.0%). For this calculation the peak area not larger than 1/5 times the peak area of propyl parahydroxybenzoate from the standard solution is excluded (0.1%).

Operating conditions—

Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay.

Time span of measurement: 2.5 times as long as the retention time of propyl parahydroxybenzoate.

System suitability—

System performance: Proceed as directed in the system suitability in the Assay.

◇Test for required detectability: To exactly 2 mL of the standard solution add the mobile phase to make exactly 10 mL. Confirm that the peak area of propyl parahydroxybenzoate obtained with 10 μL of this solution is equivalent to 14 to 26% of that with 10 μL of the standard solution.◇

◇System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of propyl parahydroxybenzoate is not more than 2.0%.◇

Residue on ignition ⟨*2.44*⟩ Not more than 0.1% (1 g).

Assay Weigh accurately about 50 mg each of Propyl Parahydroxybenzoate and Propyl Parahydroxybenzoate RS, dissolve separately in 2.5 mL each of methanol, and add the mobile phase to make exactly 50 mL. Pipet 10 mL each of these solutions, add the mobile phase to make exactly 100 mL, and use these solutions as the sample solution and the standard solution, respectively. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, and determine the peak areas, A_T and A_S, of propyl parahydroxybenzoate in each solution.

Amount (mg) of propyl parahydroxybenzoate ($C_{10}H_{12}O_3$) $= M_S \times A_T/A_S$

M_S: Amount (mg) of Propyl Parahydroxybenzoate RS taken

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 272 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

◇Column temperature: A constant temperature of about

35°C.◇

Mobile phase: A mixture of methanol and potassium dihydrogen phosphate solution (17 in 2500) (13:7).

Flow rate: 1.3 mL per minute.

System suitability—

System performance: Dissolve 5 mg each of Propyl Parahydroxybenzoate, ethyl parahydroxybenzoate and parahydroxybenzoic acid in the mobile phase to make exactly 100 mL. Pipet 1 mL of this solution, and add the mobile phase to make exactly 10 mL. When the procedure is run with 10 μL of this solution under the above operating conditions, parahydroxybenzoic acid, ethyl parahydroxybenzoate and propyl parahydroxybenzoate are eluted in this order, the relative retention times of parahydroxybenzoic acid and ethyl parahydroxybenzoate to propyl parahydroxybenzoate are about 0.3 and about 0.7, respectively, and the resolution between the peaks of ethyl parahydroxybenzoate and propyl parahydroxybenzoate is not less than 3.0.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of propyl parahydroxybenzoate is not more than 0.85%.

◆**Containers and storage** Containers—Well-closed containers.◆

Sarpogrelate Hydrochloride Fine Granules

サルポグレラート塩酸塩細粒

Change the Uniformity of dosage units and Assay as follows:

Uniformity of dosage units ⟨*6.02*⟩ Perform the test according to the following method: Sarpogrelate Hydrochloride Fine Granules in single-dose packages meet the requirement of the Content uniformity test.

To the total amount of the content of 1 package of Sarpogrelate Hydrochloride Fine Granules add $4V/5$ mL of the mobile phase, disperse the particles by sonicating, then add the mobile phase to make exactly V mL so that each mL contains about 1 mg of sarpogrelate hydrochloride ($C_{24}H_{31}NO_6$.HCl), and centrifuge. Pipet 5 mL of the supernatant liquid, add the mobile phase to make exactly 50 mL, and use this solution as the sample solution. Then, proceed as directed in the Assay.

Amount (mg) of sarpogrelate hydrochloride ($C_{24}H_{31}NO_6$.HCl)
$= M_S \times A_T/A_S \times V/50$

M_S: Amount (mg) of Sarpogrelate Hydrochloride RS taken, calculated on the anhydrous basis

Assay Powder Sarpogrelate Hydrochloride Fine Granules. Weigh accurately a portion of the powder, equivalent to about 0.25 g of sarpogrelate hydrochloride ($C_{24}H_{31}NO_6$.HCl), add 200 mL of the mobile phase, and disperse the particles by sonicating. To this solution add the mobile phase to make exactly 250 mL, and centrifuge. Pipet 5 mL of the supernatant liquid, add the mobile phase to make exactly 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 50 mg of Sarpogrelate Hydrochloride RS (separately determine the water ⟨*2.48*⟩ in the same manner as Sarpogrelate Hydroxide), and add the mobile phase to make exactly 50 mL. Pipet 5 mL of this solution, add the mobile phase to make exactly 50 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, and determine the peak areas, A_T and A_S, of sarpogrelate in each solution.

Amount (mg) of sarpogrelate hydrochloride ($C_{24}H_{31}NO_6$.HCl)
$= M_S \times A_T/A_S \times 5$

M_S: Amount (mg) of Sarpogrelate Hydrochloride RS taken, calculated on the anhydrous basis

Operating conditions—

Proceed as directed in the operating conditions in the Assay under Sarpogrelate Hydrochloride.

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of sarpogrelate are not less than 5000 and not more than 1.8, respectively.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of sarpogrelate is not more than 1.0%.

Sodium Chloride

塩化ナトリウム

Change the Identification as follows:

Identification **(1)** A solution of Sodium Chloride (1 in 20) responds to Qualitative Tests (2) ⟨*1.09*⟩ for sodium salt.

(2) A solution of Sodium Chloride (1 in 20) responds to Qualitative Tests (2) ⟨*1.09*⟩ for chloride.

Spectinomycin Hydrochloride for Injection

注射用スペクチノマイシン塩酸塩

Change the description of Uniformity of dosage units as follows:

Uniformity of dosage units ⟨*6.02*⟩ It meets the requirement of the Mass variation test (*T*: Being specified separately when the drug is granted approval based on the Law.).

Wheat Starch

コムギデンプン

Change the Purity (5) as follows:

Purity

(5) Total protein—Weigh accurately about 3 g of Wheat Starch, place it in a Kjeldahl flask, add 4 g of a decomposition accelerator (a powdered mixture of 100 g of potassium sulfate, 3 g of copper (II) sulfate pentahydrate and 3 g of titanium (IV) oxide), wash down any adhering substances from the neck of the flask with a fine jet of water. Add 25 mL of sulfuric acid allowing to flow down the inside wall of the flask, and mix the contents. Close the mouth of the flask loosely, for example by means of a glass bulb with a short stem, to avoid excessive loss of the sulfuric acid. Heat the flask gradually at first, then increase the temperature until there is vigorous boiling with condensation of sulfuric acid in the neck of the flask, preventing the upper part of the flask from becoming overheated. Continue the heating until the solution becomes clear, and the inside wall of the flask is free from a carbonaceous material. After cooling, dissolve the solid material by adding cautiously 25 mL of water, cool again, and place in a steam-distillation apparatus previously washed by passing steam. Add exactly 25 mL of 0.01 mol/L hydrochloric acid VS and a suitable amount of water into the receiver, and immerse the tip of the condenser in this acid solution. Add the same quantity of a solution of sodium hydroxide (21 in 50) as used for a blank determination through the funnel, and distill immediately by passing steam through the mixture. Collect about 40 mL of distillate, lower the receiver so that the tip of the condenser is above the surface of the acid solution, then continue the distillation for a while, and rinse the end part of the condenser with a small amount of water. Titrate ⟨*2.50*⟩ the excessive hydrochloric acid with 0.025 mol/L sodium hydroxide VS until the color of the solution changes from red-purple through grayish blue to green (indicator: 3 drops of methyl red-methylene blue TS). Perform a blank determination in the same manner. The amount of a solution of sodium hydroxide (21 in 50) to be added from the funnel is sufficient to change the color of the solution in the flask from bluish green to dark brown or black.

$$\text{Amount (\%) of nitrogen} = (a-b) \times 0.035/M$$

M: Amount (g) of Wheat Starch taken
a: Volume (mL) of 0.025 mol/L sodium hydroxide VS consumed in a blank determination
b: Volume (mL) of 0.025 mol/L sodium hydroxide VS consumed in the sample determination

The amount of total protein is not more than 0.3% [0.048% as nitrogen (N: 14.01) (using conversion factor of nitrogen to protein, 6.25)].

Stearic Acid

ステアリン酸

Change the Congealing point as follows:

Congealing point The apparatus consists of a test tube about 25 mm in diameter and 150 mm long placed inside a test tube about 40 mm in diameter and 160 mm long. The inner tube is closed by a stopper which carries a thermometer about 175 mm long and graduated in 0.2°C fixed so that ◆the upper end of◆ the bulb is about 15 mm above the bottom of the tube. The stopper has a hole allowing the passage of the stem of a stirrer made from a glass rod or other suitable material formed at one end into a loop of about 18 mm overall diameter at right angles to the rod. The inner tube with its jacket is supported centrally in a 1-L beaker containing a suitable cooling liquid to within 20 mm of the top. A thermometer is supported in the cooling bath. Place in the inner tube sufficient quantity of the liquid or previously melted substance to be examined, to cover the thermometer bulb and determine the approximate congealing point by cooling rapidly. Place the inner tube in a bath about 5°C above the approximate congealing point until all but the last traces of crystals are melted. Fill the beaker with water or a saturated solution of sodium chloride, at a temperature about 5°C lower than the expected congealing point, insert the inner tube into the outer tube, ensuring that some seed crystals are present, and stir thoroughly until solidification takes place. Note the highest temperature observed during solidification.

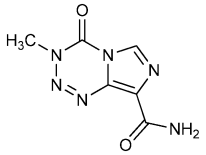
The apparatus directed under Congealing Point Determination ⟨*2.42*⟩ can also be used. Transfer the melted sample into sample container B up to the marked line C. Adjust the immersion line H of thermometer F to the same level of the meniscus of the sample, and then determine the approximate congealing point by cooling rapidly. Place the sample container B in a bath at a temperature about 5°C above the approximate congealing point until all but the last traces of crystals are melted. Fill bath D with water or a saturated solution of sodium chloride, at a temperature about 5°C lower than the expected congealing point, and set the sample container B in A. Ensuring that some seed crystals are present, stir thoroughly until solidification takes place. Note the highest temperature observed during solidification.

The congealing point of stearic acid 50 is 53 – 59°C, of stearic acid 70 is 57 – 64°C, and of stearic acid 95 is 64 – 69°C.

Add the following:

Temozolomide

テモゾロミド



$C_6H_6N_6O_2$: 194.15

3-Methyl-4-oxo-3,4-dihydroimidazo[5,1-*d*][1,2,3,5]tetrazine-8-carboxamide

[*85622-93-1*]

Temozolomide contains not less than 98.0% and not more than 102.0% of temozolomide ($C_6H_6N_6O_2$).

Description Temozolomide occurs as a white to pale red or light yellow-brown, crystalline powder or powder.

It is sparingly soluble in dimethyl sulfoxide, slightly soluble in water and in acetonitrile, and very slightly soluble in ethanol (99.5).

Melting point: 180°C (with decomposition).

It shows crystal polymorphism.

Identification **(1)** Determine the absorption spectrum of a solution of Temozolomide (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Temozolomide RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Temozolomide as directed in the potassium bromide disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum or the spectrum of Temozolomide RS: both spectra exhibit similar intensities of absorption at the same wave numbers. If any difference appears between the spectra, dissolve Temozolomide in acetonitrile, evaporate the acetonitrile, dry the residue, and perform the test with the residue.

Purity **(1)** Related substances—Use the sample solution obtained in the Assay as the sample solution. Pipet 1 mL of the sample solution, add dimethyl sulfoxide to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine each peak area by the automatic integration method: the peak area of the related substance E, having the relative retention time of about 0.4 to temozolomide, obtained from the sample solution is not larger than 1/5 times the peak area of temozolomide from the standard solution, the peak area of the related substance D, having the relative retention time of about 0.5, from the sample solution is not larger than 1/2 times the peak area of temozolomide from the standard solution, and the area of the peak other than temozolomide and the peaks mentioned above from the sample solution is not larger than 1/10 times the peak area of temozolomide from the standard solution. Furthermore, the total area of the peaks other than temozolomide from the sample solution is not larger than 4/5 times the peak area of temozolomide from the standard solution. For the peak area of the related substance E, multiply the correction factor 0.63.

Operating conditions—

Detector, column, column temperature, mobile phase and flow rate: Proceed as directed in the operating conditions in the Assay.

Time span of measurement: About 3 times as long as the retention time of temozolomide, beginning after the solvent peak.

System suitability—

System performance: Proceed as directed in the system suitability in the Assay.

Test for required detectability: Pipet 1 mL of the standard solution, and add dimethyl sulfoxide to make exactly 20 mL. Confirm that the peak area of temozolomide obtained with 10 μL of this solution is equivalent to 3.5 to 6.5% of that with 10 μL of the standard solution.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of temozolomide is not more than 2.0%.

(2) Residual solvent—Being specified separately when the drug is granted approval based on the Law.

Water <*2.48*> Not more than 0.4% (0.5 g, coulometric titration).

Residue on ignition <*2.44*> Not more than 0.1% (1 g).

Assay Weigh accurately about 25 mg each of Temozolomide and Temozolomide RS, to each add 20 mL of dimethyl sulfoxide, shake to dissolve, add dimethyl sulfoxide to make exactly 25 mL, and use these solutions as the sample solution and the standard solution, respectively. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine the peak areas, A_T and A_S, of temozolomide in each solution.

$$\text{Amount (mg) of temozolomide } (C_6H_6N_6O_2) = M_S \times A_T/A_S$$

M_S: Amount (mg) of Temozolomide RS taken

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 270 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized

silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: To 5 mL of acetic acid (100) add 1000 mL of water. To 24 volumes of this solution add 1 volume of methanol. Dissolve 0.94 g of sodium 1-hexanesulfonate in 1000 mL of this solution.

Flow rate: Adjust so that the retention time of temozolomide is about 9.5 minutes.

System suitability—

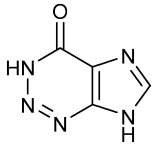
System performance: To 5 mL of the sample solution add 5 mL of 0.1 mol/L hydrochloric acid TS, heat on a water bath for 1 hour, and cool to 4°C. When the procedure is run with 10 μL of this solution under the above operating conditions, the resolution between the peak of temozolomide and the peak having the relative retention time of about 1.4 to temozolomide is not less than 2.5, and the symmetry factor of the peak of temozolomide is not more than 1.9.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of temozolomide is not more than 1.0%.

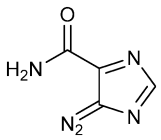
Containers and storage Containers—Well-closed containers (moisture-proof packaging).

Others

Related substance E:
3,7-Dihydro-4*H*-imidazo[4,5-*d*][1,2,3]triazin-4-one



Related substance D:
4-Diazo-4*H*-imidazole-5-carboxamide



Add the following:

Temozolomide Capsules

テモゾロミドカプセル

Temozolomide Capsules contain not less than 95.0% and not more than 105.0% of the labeled amount of temozolomide ($C_6H_6N_6O_2$: 194.15).

Method of preparation Prepare as directed under Capsules, with Temozolomide.

Identification Perform the test with 20 μL each of the sample solution and standard solution obtained in the Assay as directed under Liquid Chromatography <*2.01*> according to the following conditions: the retention times of the principal peaks in the chromatograms obtained from these solutions are the same, and the absorption spectra of these peaks exhibit similar intensities of absorption at the same wavelengths.

Operating conditions—

Column, column temperature, mobile phase and flow rate: Proceed as directed in the operating conditions in the Assay.

Detector: A photodiode array detector (wavelength: 270 nm, spectrum range of measurement: 210 – 400 nm).

System suitability—

System performance: Proceed as directed in the system suitability in the Assay.

Purity Related substances—Use the sample solution obtained in the Assay as the sample solution. Pipet 1 mL of the sample solution, add dimethyl sulfoxide to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine each peak area by the automatic integration method: the peak area of the related substance E, having the relative retention time of about 0.4 to temozolomide, obtained from the sample solution is not larger than 3/5 times the peak area of temozolomide from the standard solution, the peak area of the related substance CA, having the relative retention time of about 1.4, from the sample solution is not larger than the peak area of temozolomide from the standard solution, and the area of the peak other than temozolomide and the peaks mentioned above from the sample solution is not larger than 1/5 times the peak area of temozolomide from the standard solution. Furthermore, the total area of the peaks other than temozolomide from the sample solution is not larger than 1.2 times the peak area of temozolomide from the standard solution. For the peak areas of the related substances E and CA, multiply the correction factors, 0.63 and 0.30, respectively.

Operating conditions—

Detector, Column, column temperature, mobile phase and flow rate: Proceed as directed in the operating conditions in the Assay under Temozolomide.

Time span of measurement: About 3 times as long as the retention time of temozolomide, beginning after the solvent peak.

System suitability—

System performance: Proceed as directed in the system suitability in the Assay.

Test for required detectability: Pipet 2 mL of the standard solution, and add the mobile phase to make exactly 20 mL. Confirm that the peak area of temozolomide obtained with 20 μL of this solution is equivalent to 7 to 13% of that with 20 μL of the standard solution.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operat-

ing conditions, the relative standard deviation of the peak area of temozolomide is not more than 2.0%.

Uniformity of dosage units ⟨*6.02*⟩ Perform the Mass variation test, or the Content uniformity test according to the following method: it meets the requirement.

To 1 capsule of Temozolomide Capsules add exactly V mL of the mobile phase so that each mL contains about 1 mg of temozolomide ($C_6H_6N_6O_2$), and shake until the capsule is completely disintegrated. Shake until the content is dispersed, centrifuge for 10 minutes, and filter the supernatant liquid through a membrane filter with a pore size of 0.45 μm. Discard the first 3 mL of the filtrate, pipet 10 mL of the subsequent filtrate, add the mobile phase to make exactly 100 mL, and use this solution as the sample solution. Then, proceed as directed in the Assay.

Amount (mg) of temozolomide ($C_6H_6N_6O_2$)
$= M_S \times A_T/A_S \times V/25$

M_S: Amount (mg) of Temozolomide RS taken

Dissolution ⟨*6.10*⟩ When the test is performed at 100 revolutions per minute according to the Basket method, using 900 mL of water as the dissolution medium, the Q value in 30 minutes of Temozolomide Capsules is 80%.

Start the test with 1 capsule of Temozolomide Capsules, withdraw not less than 10 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.8 μm. Discard not less than 3 mL of the first filtrate, pipet V mL of the subsequent filtrate, add water to make V' mL so that each mL contains about 22 μg of temozolomide ($C_6H_6N_6O_2$), and use this solution as the sample solution. Separately, weigh accurately about 22 mg of Temozolomide RS, and dissolve in water to make exactly 100 mL. Pipet 10 mL of this solution, add water to make exactly 100 mL, and use this solution as the standard solution. Determine the absorbances, A_T and A_S, of the sample solution and standard solution at 328 nm as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩.

Dissolution rate (%) with respect to the labeled amount of temozolomide ($C_6H_6N_6O_2$)
$= M_S \times A_T/A_S \times V'/V \times 1/C \times 90$

M_S: Amount (mg) of Temozolomide RS taken
C: Labeled amount (mg) of temozolomide ($C_6H_6N_6O_2$) in 1 capsule

Assay To 10 Temozolomide Capsules add the mobile phase, and shake until the capsules are completely disintegrated. Shake until the content is dispersed, and add the mobile phase to make exactly V mL so that each mL contains about 1 mg of temozolomide ($C_6H_6N_6O_2$). Centrifuge this solution for 10 minutes, and filter the supernatant liquid through a membrane filter with a pore size of 0.45 μm. Discard the first 3 mL of the filtrate, pipet 10 mL of the subsequent filtrate, add the mobile phase to make exactly 100 mL, and use this solution as the sample solution. Separately, weigh accurately about 25 mg of Temozolomide RS, add 200 mL of the mobile phase, sonicate to dissolve, add the mobile phase to make exactly 250 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, and determine the peak areas, A_T and A_S, of temozolomide in each solution.

Amount (mg) of temozolomide ($C_6H_6N_6O_2$) in 1 capsule
$= M_S \times A_T/A_S \times V/250$

M_S: Amount (mg) of Temozolomide RS taken

Operating conditions—

Proceed as directed in the operating conditions in the Assay under Temozolomide.

System suitability—

System performance: Dissolve 10 mg of temozolomide in 25 mL of the mobile phase. To this solution add 25 mL of 0.1 mol/L hydrochloric acid TS, allow to stand at 80°C for 4 hours, cool to 4°C, and preserve. When the procedure is run with 20 μL of this solution under the above operating conditions, the resolution between the peaks of temozolomide and the related substance CA is not less than 2.5, and the symmetry factor of the peak of temozolomide is not more than 1.9.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of temozolomide is not more than 1.0%.

Containers and storage Containers—Tight containers.

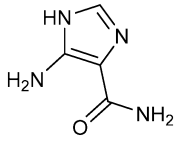
Others

Related substance E:

Refer to it described in Temozolomide.

Related substance CA:

5-Amino-1*H*-imidazole-4-carboxamide



Add the following:

Temozolomide for Injection

注射用テモゾロミド

Temozolomide for Injection is a preparation for injection which is dissolved before use.

It contains not less than 95.0% and not more than 105.0% of the labeled amount of temozolomide ($C_6H_6N_6O_2$: 194.15).

Method of preparation Prepare as directed under Injections, with Temozolomide.

Description Temozolomide for Injection occurs as a white

to pale red or light yellow-brown powder.

Identification Perform the test with 75 μL each of the sample solution and standard solution obtained in the Assay as directed under Liquid Chromatography <*2.01*> according to the following conditions: the retention times of the principal peaks in the chromatograms obtained from these solutions are the same, and the absorption spectra of these peaks exhibit similar intensities of absorption at the same wavelengths.

Operating conditions—

Column, column temperature, mobile phase and flow rate: Proceed as directed in the operating conditions in the Assay under Temozolomide.

Detector: A photodiode array detector (wavelength: 270 nm, spectrum range of measurement: 210 – 400 nm).

System suitability—

System performance: Proceed as directed in the system suitability in the Assay.

pH Being specified separately when the drug is granted approval based on the Law.

Purity Related substances—Use the sample solution obtained in the Assay as the sample solution. Pipet 1 mL of the sample solution, add the mobile phase to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 75 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine each peak area by the automatic integration method: the peak area of the related substance E, having the relative retention time of about 0.4 to temozolomide, obtained from the sample solution is not larger than 2/5 times the peak area of temozolomide from the standard solution, the peak area of the related substance IA, having the relative retention time of about 1.4, from the sample solution is not larger than the peak area of temozolomide from the standard solution, and the area of the peak other than temozolomide and the peaks mentioned above from the sample solution is not larger than 1/5 times the peak area of temozolomide from the standard solution. Furthermore, the total area of the peaks other than temozolomide from the sample solution is not larger than the peak area of temozolomide from the standard solution. For the peak areas of the related substances E and IA, multiply the correction factors, 0.63 and 0.29, respectively.

Operating conditions—

Detector, Column, column temperature, mobile phase and flow rate: Proceed as directed in the operating conditions in the Assay under Temozolomide.

Time span of measurement: About 3 times as long as the retention time of temozolomide, beginning after the solvent peak.

System suitability—

System performance: Proceed as directed in the system suitability in the Assay.

Test for required detectability: Pipet 5 mL of the standard solution obtained in the Assay, and add the mobile phase to make exactly 200 mL. Pipet 2 mL of this solution, and add the mobile phase to make exactly 100 mL. When the procedure is run with 75 μL of this solution under the above operating conditions, the SN ratio of the peak of temozolomide is not less than 10.

System repeatability: When the test is repeated 6 times with 75 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of temozolomide is not more than 2.0%.

Water <*2.48*> To an amount of Temozolomide for Injection, equivalent to 100 mg of Temozolomide, add exactly 40 mL of methanol to dissolve the content, pipet 2 mL of the solution, and perform the test by coulometric titration: not more than 1.0%. Perform a blank determination in the same manner, and make any necessary correction.

Bacterial endotoxins <*4.01*> Less than 0.75 EU/mg.

Uniformity of dosage units <*6.02*> It meets the requirement of the Mass variation test (*T*: being specified separately when the drug is granted approval based on the Law.).

Foreign insoluble matter <*6.06*> Perform the test according to Method 2: it meets the requirement.

Insoluble particulate matter <*6.07*> It meets the requirement.

Sterility <*4.06*> Perform the test according to the Membrane filtration method: it meets the requirement.

Assay Take a number of Temozolomide for Injection, equivalent to 500 mg of temozolomide ($C_6H_6N_6O_2$), dissolve each content in water, wash each container with water, combine the washings with the former solution, and add water to the combined solution to make exactly 200 mL. Pipet 5 mL of this solution, add the mobile phase to make exactly 100 mL, and use this solution as the sample solution. Separately, weigh accurately about 31 mg of Temozolomide RS, and add the mobile phase to make exactly 50 mL. Pipet 10 mL of this solution, add the mobile phase to make exactly 50 mL, and use this solution as the standard solution. Perform the test with exactly 75 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine the peak areas, A_T and A_S, of temozolomide in each solution.

$$\text{Amount (mg) of temozolomide } (C_6H_6N_6O_2) = M_S \times A_T/A_S \times 16$$

M_S: Amount (mg) of Temozolomide RS taken

Operating conditions—

Proceed as directed in the operating conditions in the Assay under Temozolomide.

System suitability—

System performance: To 1 mg of temozolomide add a mixture of the mobile phase and 0.1 mol/L hydrochloric acid TS (1:1) to make 10 mL, heat at 80°C for about 4 hours, and cool to about 4°C. To this solution add the mo-

bile phase to make 25 mL. When the procedure is run with 75 μL of this solution under the above operating conditions, the resolution between the peaks of temozolomide and the related substance IA is not less than 2.5, and the symmetry factor of the peak of temozolomide is not more than 1.9.

System repeatability: When the test is repeated 6 times with 75 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of temozolomide is not more than 1.0%.

Containers and storage Containers—Hermetic containers.

Storage—At a temperature between 2°C and 8°C.

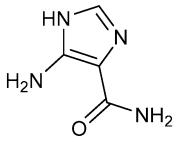
Others

Related substance E:

Refer to it described in Temozolomide.

Related substance IA:

5-Amino-1*H*-imidazole-4-carboxamide



Add the following:

Voglibose Orally Disintegrating Tablets

ボグリボースロ腔内崩壊錠

Voglibose Orally Disintegrating Tablets contain not less than 95.0% and not more than 105.0% of the labeled amount of voglibose ($C_{10}H_{21}NO_7$: 267.28).

Method of preparation Prepare as directed under Tablets, with Voglibose.

Identification To 10 tablets of Voglibose Orally Disintegrating Tablets, crushed if necessary, add methanol so that each mL contains about 0.2 mg of voglibose ($C_{10}H_{21}NO_7$), sonicate while shaking to disintegrate the tablets. Filter this solution through a membrane filter with a pore size not exceeding 0.45 μm, discard the first 3 mL of the filtrate, and use the subsequent filtrate as the sample solution. Separately, dissolve 10 mg of voglibose for assay in 2 mL of water, add methanol to make 50 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of methanol, acetone, water and ammonia solution (28) (10:10:4:1) to a distance of about 12 cm, and air-dry the plate. Then, immerse the plate in lead tetraacetate-fluorescein sodium TS, and lift gently to allow the excessive solution to flow out. After air-drying, examine under ultraviolet light (main wavelength: 366 nm): the spots obtained from the sample solution and standard solution exhibit a yellow fluorescence and show the same *R*f value.

Uniformity of dosage units <*6.02*> Perform the test according to the following method: it meets the requirement of the Content uniformity test.

To 1 tablet of Voglibose Orally Disintegrating Tablets add exactly *V* mL of the mobile phase so that each mL contains about 20 μg of voglibose ($C_{10}H_{21}NO_7$), and sonicate to disintegrate the tablet. Centrifuge this solution, and filter the supernatant liquid through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 5 mL of the filtrate, and use the subsequent filtrate as the sample solution. Then, proceed as directed in the Assay.

Amount (mg) of voglibose ($C_{10}H_{21}NO_7$)
$= M_S \times A_T/A_S \times V/2500$

M_S: Amount (mg) of voglibose for assay taken, calculated on the anhydrous basis

Disintegration Being specified separately when the drug is granted approval based on the Law.

Dissolution <*6.10*> When the test is performed at 50 revolutions per minute according to the Paddle method, using 900 mL of water as the dissolution medium, the dissolution rate in 15 minutes of Voglibose Orally Disintegrating Tablets is not less than 85%.

Start the test with 1 tablet of Voglibose Orally Disintegrating Tablets, withdraw not less than 10 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard not less than 5 mL of the first filtrate, pipet *V* mL of the subsequent filtrate, add the mobile phase to make exactly *V′* mL so that each mL contains about 0.11 μg of voglibose ($C_{10}H_{21}NO_7$), and use this solution as the sample solution. Separately, weigh accurately about 50 mg of voglibose for assay (separately determine the water <*2.48*> in the same manner as Voglibose), and dissolve in water to make exactly 50 mL. Pipet 1 mL of this solution, and add water to make exactly 100 mL. Pipet 2 mL of this solution, and add water to make exactly 100 mL. Pipet 10 mL of this solution, add the mobile phase to make exactly 20 mL, and use this solution as the standard solution. Perform the test with 100 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine the peak areas, A_T and A_S, of voglibose in each solution.

Dissolution rate (%) with respect to the labeled amount of voglibose ($C_{10}H_{21}NO_7$)
$= M_S \times A_T/A_S \times V'/V \times 1/C \times 9/50$

M_S: Amount (mg) of voglibose for assay taken, calculated on the anhydrous basis

C: Labeled amount (mg) of voglibose ($C_{10}H_{21}NO_7$) in 1 tablet

Operating conditions—

Apparatus, detector, column temperature, reaction coil,

cooling coil, mobile phase, reaction reagent, reaction temperature, cooling temperature and flow rate of reaction reagent: Proceed as directed in the operating conditions in the Assay.

Column: A stainless steel column 4.6 mm in inside diameter and 7.5 cm in length, packed with polyamine silica gel for liquid chromatography (5 μm in particle diameter).

Flow rate of mobile phase: Adjust so that the retention time of voglibose is about 5 minutes.

System suitability—

System performance: When the procedure is run with 100 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of voglibose are not less than 900 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 100 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of voglibose is not more than 3.0%.

Assay To 20 tablets of Voglibose Orally Disintegrating Tablets add $4V/5$ mL of the mobile phase, and sonicate to disintegrate the tablets. Add the mobile phase to make exactly V mL so that each mL contains about 20 μg of voglibose ($C_{10}H_{21}NO_7$). Centrifuge this solution, and filter the supernatant liquid through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 5 mL of the filtrate, and use the subsequent filtrate as the sample solution. Separately, weigh accurately about 50 mg of voglibose for assay (separately determine the water <2.48> in the same manner as Voglibose), and dissolve in the mobile phase to make exactly 100 mL. Pipet 2 mL of this solution, add the mobile phase to make exactly 50 mL, and use this solution as the standard solution. Perform the test with 50 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the peak areas, A_T and A_S, of voglibose in each solution.

$$\text{Amount (mg) of voglibose } (C_{10}H_{21}NO_7) \text{ in 1 tablet} = M_S \times A_T/A_S \times V/50000$$

M_S: Amount (mg) of voglibose for assay taken, calculated on the anhydrous basis

Operating conditions—

Apparatus: Use an apparatus consisting of 2 pumps for the mobile phase and reaction reagent transportation, sample injection port, column, reaction coil, cooling coil, detector and recording device, and the reaction coil and cooling coil maintained at a constant temperature.

Detector: A fluorophotometer (excitation wavelength: 350 nm, fluorescence wavelength: 430 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with polyamine silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Reaction coil: A polytetrafluoroethylene tube 0.5 mm in inside diameter and 20 m in length.

Cooling coil: A polytetrafluoroethylene tube 0.3 mm in inside diameter and 2 m in length.

Mobile phase: Dissolve 1.56 g of sodium dihydrogen phosphate dihydrate in 500 mL of water, and adjust to pH 6.5 with a solution prepared by dissolving 3.58 g of disodium hydrogen phosphate dodecahydrate in 500 mL of water. To 500 mL of this solution add 500 mL of acetonitrile.

Reaction reagent: Dissolve 6.25 g of taurine and 2.56 g of sodium periodate in water to make 1000 mL.

Reaction temperature: A constant temperature of about 100°C.

Cooling temperature: A constant temperature of about 25°C.

Flow rate of mobile phase: Adjust so that the retention time of voglibose is about 15 minutes.

Flow rate of reaction reagent: Same as the flow rate of the mobile phase.

System suitability—

System performance: When the procedure is run with 50 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of voglibose are not less than 3000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 50 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of voglibose is not more than 1.0%.

Containers and storage Containers—Tight containers.

Voglibose Tablets

ボグリボース錠

Change the Identification as follows:

Identification Shake vigorously an amount of powdered Voglibose Tablets, equivalent to 5 mg of Voglibose, with 40 mL of water, and centrifuge. Transfer the supernatant liquid to a chromatographic column [prepared by pouring 1.0 mL of strongly acidic ion-exchange resin (H type) for column chromatography (70 to 200 μm in particle diameter) into a chromatographic column 8 mm in inside diameter and 130 mm in height], and allow to flow at a rate of about 5 mL per minute. Then wash the column with 200 mL of water, and allow to flow with 10 mL of diluted ammonia TS (1 in 4) at a rate of about 5 mL per minute. Filter the effluent solution 2 times through a membrane filter with a pore size not exceeding 0.22 μm. Evaporate the filtrate to dryness at 50°C under reduced pressure, dissolve the residue with 0.5 mL of a mixture of water and methanol (1:1), and use this solution as the sample solution. Separately, dissolve 20 mg of voglibose for assay in 2 mL of the mixture of water and methanol (1:1), and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 20 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop

the plate with a mixture of acetone, ammonia water (28) and water (5:3:1) to a distance of about 12 cm, air-dry the plate, and allow to stand in iodine vapors: the principal spot obtained from the sample solution and the spot from the standard solution show a yellow-brown color, and the same *R*f value.

Crude Drugs and Related Drugs

Achyranthes Root

ゴシツ

Change the Identification as follows:

Identification **(1)** Shake vigorously 0.5 g of pulverized Achyranthes Root with 10 mL of water: a lasting fine foam is produced.

(2) To 1.0 g of pulverized Achyranthes Root add 10 mL of methanol, shake for 10 minutes, centrifuge, and use the supernatant liquid as the sample solution. Perform the test with the sample solution as directed under Thin-layer Chromatography <*2.03*>. Spot 10 μL of the sample solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, methanol, water and acetic acid (100) (14:4:1:1) to a distance of about 7 cm, and air-dry the plate. Spray evenly 4-dimethylaminobenzaldehyde TS for spraying on the plate, heat the plate at 105°C for 5 minutes, allow to cool, and spray water: a light red to red-orange spot appears at an *R*f value of about 0.5.

Akebia Stem

モクツウ

Change the origin/limits of content as follows:

Akebia Stem is the climbing stem of *Akebia quinata* Decaisne, *Akebia trifoliata* Koidzumi, or their interspecific hybrids (*Lardizabalaceae*), usually cut transversely.

Apricot Kernel

キョウニン

Change the Assay as follows:

Assay Weigh accurately 0.5 g of ground Apricot Kernel, add 40 mL of diluted methanol (9 in 10), heat immediately under a reflux condenser for 30 minutes, and cool. Filter the mixture, add diluted methanol (9 in 10) to make exactly 50 mL. Pipet 5 mL of this solution, add water to make exactly 10 mL, and use this solution as the sample solution. Separately, weigh accurately about 10 mg of amygdalin for assay, dissolve in diluted methanol (1 in 2) to make exactly 50 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine the peak areas, A_T and A_S, of amygdalin in each solution.

$$\text{Amount (mg) of amygdalin} = M_S \times A_T/A_S \times 2$$

M_S: Amount (mg) of amygdalin for assay taken

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 210 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 45°C.

Mobile phase: A mixture of 0.05 mol/L sodium dihydrogen phosphate TS and methanol (5:1).

Flow rate: 0.8 mL per minute (the retention time of amygdalin is about 12 minutes).

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of amygdalin are not less than 5000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of amygdalin is not more than 1.5%.

Artemisia Capillaris Flower

インチンコウ

Change the Description as follows (No effect to English text):

Artemisia Leaf

ガイヨウ

Change the Description as follows (No effect to English text):

Bearberry Leaf

ウワウルシ

Change the Description and Assay as follows (No effect to English text for Description):

Assay Weigh accurately about 0.5 g of pulverized Bearberry Leaf in a glass-stoppered centrifuge tube, add 40 mL of water, shake for 30 minutes, centrifuge, and separate the supernatant liquid. To the residue add 40 mL of water, and proceed in the same manner. To the combined extracts add water to make exactly 100 mL, and use this solution as the sample solution. Separately, weigh accurately about 40 mg of arbutin for assay, dissolve in water to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions. Determine the peak areas, A_T and A_S, of arbutin in each solution.

$$\text{Amount (mg) of arbutin} = M_S \times A_T/A_S$$

M_S: Amount (mg) of arbutin for assay taken

Operating conditions—

Detector: An ultraviolet spectrophotometer (wavelength: 280 nm).

Column: A stainless steel column 4 – 6 mm in inside diameter and 15 – 25 cm in length, packed with octadecylsilanized silica gel (5 – 10 μm in particle diameter).

Column temperature: A constant temperature of about 20°C.

Mobile phase: A mixture of water, methanol and 0.1 mol/L hydrochloric acid TS (94:5:1).

Flow rate: Adjust so that the retention time of arbutin is about 6 minutes.

System suitability—

System performance: Dissolve 50 mg each of arbutin for assay, hydroquinone and gallic acid in water to make 100 mL. When the procedure is run with 10 μL of this solution under the above operating conditions, arbutin, hydroquinone and gallic acid are eluted in this order with the resolutions among these peaks being not less than 1.5.

System repeatability: When the test is repeated 5 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of arbutin is not more than 1.5%.

Bitter Cardamon

ヤクチ

Add the following next to the Description:

Identification To 1.0 g of pulverized Bitter Cardamon add 6 mL of a mixture of water and methanol (1:1) and 3 mL of hexane, shake for 5 minutes, centrifuge, and use the upper layer of the supernatant liquid as the sample solution. Separately, dissolve 1 mg of nootkatone for thin-layer chromatography in 1 mL of hexane, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 20 μL of the sample solution and 10 μL of the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of hexane and ethyl acetate (3:1) to a distance of about 7 cm, and air-dry the plate. Spray evenly 2,4-dinitrophenylhydrazine TS on the plate: one of the several spots obtained from the sample solution has the same color tone and *R*f value with the spot from the standard solution.

Burdock Fruit

ゴボウシ

Change the Description as follows:

Description Burdock Fruit is slightly curved, long obovate achene, 5 – 7 mm in length, 2.0 – 3.2 mm in width, 0.8 to 1.5 mm in thickness; externally grayish brown to brown, with black spots; hollow about 1 mm in diameter at one broad end; flat, indistinct, longitudinal ridge at the other narrow end. 100 fruits weigh 1.0 – 1.5 g.

Practically odorless; taste, bitter and oily.

Under a microscope <*5.01*>, transverse section reveals an exocarp composed of an epidermis, mesocarp of slightly sclerified parenchyma, and endocarp of a single layer of stone cells; seed coat composed of radially elongated, sclerified epidermis, and parenchyma of several cellular layers; parenchymatous cells of the mesocarp contain a brown substance; stone cells of endocarp contain solitary, discrete crystals of calcium oxalate; cotyledons with oil drops, aleurone grains, and minute crystals of calcium oxalate.

Cardamon

ショウズク

Change the Japanese commonly used name as follows (No effect to English text):

Cimicifuga Rhizome

ショウマ

Change the Purity (3) as follows:

Purity

(3) Rhizome of *Astilbe* and other species—Under a microscope <*5.01*>, pulverized Cimicifuga Rhizome does not contain rosette aggregates of calcium oxalate.

Clove

チョウジ

Change the origin/limits of content as follows:

Clove is the flowering bud of *Syzygium aromaticum* Merrill et L. M. Perry (*Eugenia caryophyllata* Thunberg) (*Myrtaceae*).

Clove Oil

チョウジ油

Change the origin/limits of content as follows:

Clove Oil is the volatile oil distilled with steam from the flower buds or leaves of *Syzygium aromaticum* Merrill et L. M. Perry (*Eugenia caryophyllata* Thunberg) (*Myrtaceae*).

It contains not less than 80.0 vol% of total eugenol.

Cnidium Monnieri Fruit

ジャショウシ

Change the latin name as follows:

Cnidii Monnieri Fructus

Cornus Fruit

サンシュユ

Change the Assay as follows:

Assay Weigh accurately about 1 g of fine cuttings of Cornus Fruit (separately determine the loss on drying <*5.01*>), put in a glass-stoppered centrifuge tube, add 30 mL of diluted methanol (1 in 2), shake for 20 minutes, centrifuge, and take the supernatant liquid. To the residue add 30 mL of diluted methanol (1 in 2), and repeat the above process twice more. Combine all the extacts, add diluted methanol (1 in 2) to make exactly 100 mL, and use this solution as the sample solution. Separately, weigh accurately about 10 mg of loganin for assay, dissolve in diluted methanol (1 in 2) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine the peak areas, A_T and A_S, of loganin in each solution.

$$\text{Amount (mg) of loganin} = M_S \times A_T/A_S$$

M_S: Amount (mg) of loganin for assay taken, calculated on the basis of the content obtained by qNMR

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 238 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 50°C.

Mobile phase: A mixture of water, acetonitrile and methanol (55:4:1).

Flow rate: Adjust so that the retention time of loganin is about 25 minutes.

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of loganin are not less than 5000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of loganin is not more than 1.5%.

Corydalis Tuber

エンゴサク

Change the Assay as follows:

Assay Weigh accurately about 1 g of pulverized Corydalis Tuber, add 30 mL of a mixture of methanol and dilute hydrochloric acid (3:1), heat under a reflux condenser for 30 minutes, and filter after cooling. To the residue add 15 mL of a mixture of methanol and dilute hydrochloric acid (3:1), and repeat the above procedure. Combine all the filtrates, add a mixture of methanol and dilute hydrochloric acid (3:1) to make exactly 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 10 mg of dehydrocorydaline nitrate for assay, dissolve in a mixture of methanol and dilute hydrochloric acid (3:1) to make exactly 200 mL, and use this solution as the standard solution. Perform the test with exactly 5 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine the peak areas, A_T and A_S, of dehydrocorydaline in each solution.

$$\text{Amount (mg) of dehydrocorydaline [as dehydrocorydaline nitrate } (C_{22}H_{24}N_2O_7)] = M_S \times A_T/A_S \times 1/4$$

M_S: Amount (mg) of dehydrocorydaline nitrate for assay taken

Operating conditions—

Detector: An ultraviolet absorption photometer (wave-

length: 340 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: Dissolve 17.91 g of disodium hydrogen phosphate dodecahydrate in 970 mL of water, and adjust to pH 2.2 with phosphoric acid. To this solution add 14.05 g of sodium perchlorate, dissolve, and add water to make exactly 1000 mL. To this solution add 450 mL of acetonitrile, then dissolve 0.20 g of sodium lauryl sulfate.

Flow rate: Adjust so that the retention time of dehydrocorydaline is about 24 minutes.

System suitability—

System performance: Dissolve 1 mg each of dehydrocorydaline nitrate for assay and berberine chloride hydrate in 20 mL of a mixture of water and acetonitrile (20:9). When the procedure is run with 5 μL of this solution under the above operating conditions, berberine and dehydrocorydaline are eluted in this order with the resolution between these peaks being not less than 1.5.

System repeatability: When the test is repeated 6 times with 5 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak areas of dehydrocorydaline is not more than 1.5%.

Powdered Corydalis Tuber

エンゴサク末

Change the Assay as follows:

Assay Weigh accurately about 1 g of Powdered Corydalis Tuber, add 30 mL of a mixture of methanol and dilute hydrochloric acid (3:1), heat under a reflux condenser for 30 minutes, and filter after cooling. To the residue add 15 mL of the mixture of methanol and dilute hydrochloric acid (3:1), and proceed in the same way as above. Combine all the filtrates, add the mixture of methanol and dilute hydrochloric acid (3:1) to make exactly 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 10 mg of dehydrocorydaline nitrate for assay, dissolve in the mixture of methanol and dilute hydrochloric acid (3:1) to make exactly 200 mL, and use this solution as the standard solution. Perform the test with exactly 5 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine the peak areas, A_T and A_S, of dehydrocorydaline in each solution.

Amount (mg) of dehydrocorydaline [as dehydrocorydaline nitrate ($C_{22}H_{24}N_2O_7$)]
$= M_S \times A_T/A_S \times 1/4$

M_S: Amount (mg) of dehydrocorydaline nitrate for assay taken

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 340 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: Dissolve 17.91 g of disodium hydrogen phosphate dodecahydrate in 970 mL of water, and adjust to pH 2.2 with phosphoric acid. To this solution add 14.05 g of sodium perchlorate, dissolve, and add water to make exactly 1000 mL. Add 450 mL of acetonitrile, and dissolve 0.20 g of sodium lauryl sulfate in this solution.

Flow rate: Adjust so that the retention time of dehydrocorydaline is about 24 minutes.

System suitability—

System performance: Dissolve 1 mg of dehydrocorydaline nitrate for assay and 1 mg of berberine chloride hydrate in 20 mL of a mixture of water and acetonitrile (20:9). When the procedure is run with 5 μL of this solution under the above operating conditions, berberine and dehydrocorydaline are eluted in this order with the resolution between these peaks being not less than 1.5.

System repeatability: When the test is repeated 6 times with 5 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of dehydrocorydaline is not more than 1.5%.

Gardenia Fruit

サンシシ

Change the origin/limits of content as follows:

Gardenia Fruit is the fruit of *Gardenia jasminoides* J. Ellis (*Rubiaceae*), sometimes after being passed through hot water or steamed.

It contains not less than 2.7% of geniposide, calculated on the basis of dried material.

Ginger

ショウキョウ

Change the Assay as follows:

Assay Weigh accurately about 1 g of pulverized Ginger (separately determine the loss on drying <*5.01*>, at 105°C for 5 hours), place in a centrifuge tube, add 30 mL of a mixture of methanol and water (3:1), shake for 20 minutes, centrifuge, and separate the supernatant liquid. To the residue add 30 mL of a mixture of methanol and water (3:1), and repeat the extraction twice more. To the combined all extracts add a mixture of methanol and water (3:1) to make

exactly 100 mL, use this solution as the sample solution. Separately, weigh accurately about 5 mg of [6]-gingerol for assay, dissolve in a mixture of methanol and water (3:1) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine the peak areas, A_T and A_S, of [6]-gingerol in each solution.

Amount (mg) of [6]-gingerol = $M_S \times A_T/A_S$

M_S: Amount (mg) of [6]-gingerol for assay taken, calculated on the basis of the content obtained by qNMR

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 205 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silicagel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: A mixture of water and acetonitrile and phosphoric acid (3800:2200:1).

Flow rate: Adjust so that the retention time of [6]-gingerol is about 19 minutes.

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of [6]-gingerol are not less than 5000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of [6]-gingerol is not more than 1.5%.

Powdered Ginger

ショウキョウ末

Change the Assay as follows:

Assay Weigh accurately about 1 g of Powdered Ginger (separately determine the loss on drying <*5.01*>, at 105°C for 5 hours), place in a centrifuge tube, add 30 mL of a mixture of methanol and water (3:1), shake for 20 minutes, centrifuge, and separate the supernatant liquid. To the residue add 30 mL of a mixture of methanol and water (3:1), and repeat the extraction twice more. To the combined all extracts add a mixture of methanol and water (3:1) to make exactly 100 mL, use this solution as the sample solution. Separately, weigh accurately about 5 mg of [6]-gingerol for assay, dissolve in a mixture of methanol and water (3:1) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine the peak areas, A_T and A_S, of [6]-gingerol in each solution.

Amount (mg) of [6]-gingerol = $M_S \times A_T/A_S$

M_S: Amount (mg) of [6]-gingerol for assay taken, calculated on the basis of the content obtained by qNMR

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 205 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: A mixture of water and acetonitrile and phosphoric acid (3800:2200:1).

Flow rate: Adjust so that the retention time of [6]-gingerol is about 19 minutes.

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of [6]-gingerol are not less than 5000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of [6]-gingerol is not more than 1.5%.

Glehnia Root and Rhizome

ハマボウフウ

Change the origin/limits of content as follows:

Glehnia Root and Rhizome is the root and rhizome of *Glehnia littoralis* F. Schmidt ex Miquel (*Umbelliferae*).

Goshajinkigan Extract

牛車腎気丸エキス

Change the Assay (1) as follows:

Assay (1) Loganin—Weigh accurately about 0.5 g of the dry extract (or an amount of the viscous extract, equivalent to about 0.5 g of the dried substance), add exactly 50 mL of diluted methanol (1 in 2), shake for 15 minutes, filter, and use the filtrate as the sample solution. Separately, weigh accurately about 10 mg of loganin for assay, dissolve in diluted methanol (1 in 2) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard so-

lution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the peak areas, A_T and A_S, of loganin in each solution.

$$\text{Amount (mg) of loganin} = M_S \times A_T/A_S \times 1/2$$

M_S: Amount (mg) of loganin for assay taken, calculated on the basis of the content obtained by qNMR

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 238 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 50°C.

Mobile phase: A mixture of water, acetonitrile and methanol (55:4:1).

Flow rate: 1.2 mL per minute (the retention time of loganin is about 25 minutes).

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates and symmetry factor of the peak of loganin are not less than 5000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of loganin is not more than 1.5%.

Goshuyuto Extract

呉茱萸湯エキス

Change the Assay (2) as follows:

Assay

(2) [6]-Gingerol—Weigh accurately about 0.5 g of the dry extract (or an amount of the viscous extract, equivalent to about 0.5 g of the dried substance), add exactly 50 mL of diluted methanol (7 in 10), shake for 30 minutes, filter, and use the filtrate as the sample solution. Separately, weigh accurately about 10 mg of [6]-gingerol for assay, dissolve in methanol to make exactly 100 mL. Pipet 5 mL of this solution, add methanol to make exactly 50 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the peak areas, A_T and A_S, of [6]-gingerol in each solution.

$$\text{Amount (mg) of [6]-gingerol} = M_S \times A_T/A_S \times 1/20$$

M_S: Amount (mg) of [6]-gingerol for assay taken, calculated on the basis of the content obtained by qNMR

Operating conditions—

Detector, column, column temperature and mobile phase: Proceed as directed in the operating conditions in (1).

Flow rate: 1.0 mL per minute (the retention time of [6]-gingerol is about 14 minutes).

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of [6]-gingerol are not less than 5000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of [6]-gingerol is not more than 1.5%.

Hachimijiogan Extract

八味地黄丸エキス

Change the Assay (1) as follows:

Assay (1) Loganin—Weigh accurately about 0.5 g of the dry extract (or an amount of the viscous extract, equivalent to about 0.5 g of the dried substance), add exactly 50 mL of diluted methanol (1 in 2), shake for 15 minutes, filter, and use the filtrate as the sample solution. Separately, weigh accurately about 10 mg of loganin for assay, dissolve in diluted methanol (1 in 2) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the peak areas, A_T and A_S, of loganin in each solution.

$$\text{Amount (mg) of loganin} = M_S \times A_T/A_S \times 1/2$$

M_S: Amount (mg) of loganin for assay taken, calculated on the basis of the content obtained by qNMR

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 238 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 50°C.

Mobile phase: A mixture of water, acetonitrile and methanol (55:4:1).

Flow rate: 1.2 mL per minute (the retention time of loganin is about 25 minutes).

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates and symmetry factor of the peak of loganin are not less than 5000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of loganin is not more than 1.5%.

Hangekobokuto Extract

半夏厚朴湯エキス

Change the Assay (3) as follows:

Assay

(3) [6]-Gingerol—Weigh accurately about 0.5 g of the dry extract (or an amount of the viscous extract, equivalent to about 0.5 g of the dried substance), add exactly 50 mL of diluted methanol (7 in 10), shake for 15 minutes, filter, and use the filtrate as the sample solution. Separately, weigh accurately about 10 mg of [6]-gingerol for assay, dissolve in methanol to make exactly 100 mL. Pipet 5 mL of this solution, add methanol to make exactly 50 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine the peak areas, A_T and A_S, of [6]-gingerol in each solution.

$$\text{Amount (mg) of [6]-gingerol} = M_S \times A_T/A_S \times 1/20$$

M_S: Amount (mg) of [6]-gingerol for assay taken, calculated on the basis of the content obtained by qNMR

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 282 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 30°C.

Mobile phase: A mixture of water, acetonitrile and phosphoric acid (620:380:1).

Flow rate: 1.0 mL per minute (the retention time of [6]-gingerol is about 15 minutes).

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of [6]-gingerol are not less than 5000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of [6]-gingerol is not more than 1.5%.

Keishibukuryogan Extract

桂枝茯苓丸エキス

Change the Assay (3) as follows:

Assay

(3) Amygdalin—Weigh accurately about 0.5 g of the dry extract (or an amount of the viscous extract, equivalent to about 0.5 g of the dried substance), add exactly 50 mL of diluted methanol (1 in 2), shake for 15 minutes, filter, and use the filtrate as the sample solution. Separately, weigh accurately about 10 mg of amygdalin for assay, dissolve in diluted methanol (1 in 2) to make exactly 50 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine the peak areas, A_T and A_S, of amygdalin in each solution.

$$\text{Amount (mg) of amygdalin} = M_S \times A_T/A_S$$

M_S: Amount (mg) of amygdalin for assay taken

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 210 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silicagel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 45°C.

Mobile phase: A mixture of 0.05 mol/L sodium dihydrogen phosphate TS and methanol (5:1).

Flow rate: 0.8 mL per minute (the retention time of amygdalin is about 12 minutes).

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of amygdalin are not less than 5000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of amygdalin is not more than 1.5%.

Leonurus Herb

ヤクモソウ

Change the Description as follows (No effect to English text):

Magnolia Bark

コウボク

Change the origin/limits of content as follows:

Magnolia Bark is the bark of the trunk of *Magnolia obovata* Thunberg (*Magnolia hypoleuca* Siebold et Zuccarini), *Magnolia officinalis* Rehder et E. H. Wilson or *Magnolia officinalis* Rehder et E. H. Wilson var. *biloba* Rehder et E. H. Wilson (*Magnoliaceae*).

It contains not less than 0.8% of magnolol.

Maoto Extract

麻黄湯エキス

Change the Assay (2) as follows:

Assay

(2) Amygdalin—Weigh accurately about 0.5 g of the dry extract (or an amount of the viscous extract, equivalent to about 0.5 g of the dried substance), add exactly 50 mL of diluted methanol (1 in 2), shake for 15 minutes, and filter. Pipet 5 mL of the filtrate, flow through in a column packed with 2 g of polyamide for column chromatography, then elute with water to make exactly 20 mL, and use this effluent as the sample solution. Separately, weigh accurately about 10 mg of amygdalin for assay, dissolve in diluted methanol (1 in 2) to make exactly 50 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine the peak areas, A_T and A_S, of amygdalin in each solution.

$$\text{Amount (mg) of amygdalin} = M_S \times A_T/A_S \times 4$$

M_S: Amount (mg) of amygdalin for assay taken

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 210 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 45°C.

Mobile phase: A mixture of 0.05 mol/L sodium dihydrogen phosphate TS and methanol (5:1).

Flow rate: 0.8 mL per minute (the retention time of amygdalin is about 12 minutes).

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of amygdalin are not less than 5000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of amygdalin is not more than 1.5%.

Mukoi-Daikenchuto Extract

無コウイ大建中湯エキス

Change the Assay (2) as follows:

Assay

(2) [6]-Shogaol—Weigh accurately about 0.5 g of Mukoi-Daikenchuto Extract, add exactly 50 mL of diluted methanol (3 in 4), shake for 15 minutes, centrifuge, and use the supernatant liquid as the sample solution. Separately, weigh accurately about 10 mg of [6]-shogaol for assay, dissolve in diluted methanol (3 in 4) to make exactly 100 mL. Pipet 10 mL of this solution, add diluted methanol (3 in 4) to make exactly 50 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine the peak areas, A_T and A_S, of [6]-shogaol in each solution.

$$\text{Amount (mg) of [6]-shogaol} = M_S \times A_T/A_S \times 1/10$$

M_S: Amount (mg) of [6]-shogaol for assay taken, calculated on the basis of the content obtained by qNMR

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 225 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 50°C.

Mobile phase: Dissolve 0.1 g of oxalic acid dihydrate in 600 mL of water, and add 400 mL of acetonitrile.

Flow rate: 1.0 mL per minute (the retention time of [6]-shogaol is about 30 minutes).

System suitability—

System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of [6]-shogaol are not less than 5000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of [6]-shogaol is not more than 1.5%.

Nutmeg

ニクズク

Change the Japanese commonly used name as follows (No effect to English text):

Peach Kernel

トウニン

Change the Assay as follows:

Assay Weigh accurately 0.5 g of ground Peach Kernel, add 40 mL of diluted methanol (9 in 10), heat immediately under a reflux condenser for 30 minutes, and cool. Filter the mixture, add diluted methanol (9 in 10) to make exactly 50 mL. Pipet 5 mL of this solution, add water to make exactly 10 mL, and use this solution as the sample solution. Separately, weigh accurately about 10 mg of amygdalin for assay, dissolve in diluted methanol (1 in 2) to make exactly 50 mL, and use this solution as the standard solution.

Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine the peak areas, A_T and A_S, of amygdalin in each solution.

$$\text{Amount (mg) of amygdalin} = M_S \times A_T/A_S \times 2$$

M_S: Amount (mg) of amygdalin for assay taken

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 210 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilianized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 45°C.

Mobile phase: A mixture of 0.05 mol/L sodium dihydrogen phosphate TS and methanol (5:1).

Flow rate: 0.8 mL per minute (the retention time of amygdalin is about 12 minutes).

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of amygdalin are not less than 5000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of amygdalin is not more than 1.5%.

Powdered Peach Kernel

トウニン末

Change the Assay as follows:

Assay Weigh accurately 0.5 g of Powdered Peach Kernel, add 40 mL of diluted methanol (9 in 10), heat immediately under a reflux condenser for 30 minutes, and cool. Filter the mixture, add diluted methanol (9 in 10) to make exactly 50 mL. Pipet 5 mL of this solution, add water to make exactly 10 mL, and use this solution as the sample solution. Separately, weigh accurately about 10 mg of amygdalin for assay, dissolve in diluted methanol (1 in 2) to make exactly 50 mL, and use this solution as the standard solution. Perform the test exactly with 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine the peak areas, A_T and A_S, of amygdalin in each solution.

$$\text{Amount (mg) of amygdalin} = M_S \times A_T/A_S \times 2$$

M_S: Amount (mg) of amygdalin for assay taken

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 210 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilianized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 45°C.

Mobile phase: A mixture of 0.05 mol/L sodium dihydrogen phosphate TS and methanol (5:1).

Flow rate: 0.8 mL per minute (the retention time of amygdalin is about 12 minutes).

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of amygdalin are not less than 5000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of amygdalin is not more than 1.5%.

Picrasma Wood

ニガキ

Add the following next to the Description as follows:

Identification To 0.1 g of pulverized Picrasma Wood add 5 mL of methanol, shake for 5 minutes, filter, and use the

filtrate as the sample solution. Perform the test with the sample solution as directed under Thin-layer Chromatography <2.03>. Spot 2 μL of the sample solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate and hexane (20:1) to a distance of about 7 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 365 nm): a blue-white fluorescent spot appears at an *R*f value of about 0.35.

Powdered Picrasma Wood

ニガキ末

Add the following next to the Description as follows:

Identification To 0.1 g of Powdered Picrasma Wood add 5 mL of methanol, shake for 5 minutes, filter, and use the filtrate as the sample solution. Perform the test with the sample solution as directed under Thin-layer Chromatography <2.03>. Spot 2 μL of the sample solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate and hexane (20:1) to a distance of about 7 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 365 nm): a blue-white fluorescent spot appears at an *R*f value of about 0.35.

Plantago Herb

シャゼンソウ

Change the Description as follows (No effect to English text):

Prepared Glycyrrhiza

シャカンゾウ

Change the Description as follows:

Description Usually cut; in case with periderm external surface dark brown to dark red-brown and with longitudinal wrinkles; in case periderm fallen off, external surface light yellow-brown to brown and fibrous; on transversely cut surface light yellow-brown to brown, cortex and xylem almost distinctly defined, and exhibits radial structure; sometimes radial cleft observed.

Odor, fragrant; taste sweet, followed by slight bitterness.

Processed Ginger

カンキョウ

Change the Assay as follows:

Assay Weigh accurately about 1 g of pulverized Processed Ginger, place in a centrifuge tube, add 30 mL of the mobile phase, shake for 20 minutes, centrifuge, and separate the supernatant liquid. To the residue add 30 mL of the mobile phase, and repeat the extraction twice more. To the combined all extracts add the mobile phase to make exactly 100 mL, use this solution as the sample solution. Separately, weigh accurately about 5 mg of [6]-shogaol for assay, dissolve in the mobile phase to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the peak areas, A_T and A_S, of [6]-shogaol in each solution.

$$\text{Amount (mg) of [6]-shogaol} = M_S \times A_T/A_S$$

M_S: Amount (mg) of [6]-shogaol for assay taken, calculated on the basis of the content obtained by qNMR

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 225 nm).

Column: A stainless steel column 6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: A mixture of acetonitrile and water (3:2).

Flow rate: Adjust so that the retention time of [6]-shogaol is about 14 minutes.

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of [6]-shogaol are not less than 5000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of [6]-shogaol is not more than 1.5%.

Add the following:

Saikokeishikankyoto Extract

柴胡桂枝乾姜湯エキス

Saikokeishikankyoto Extract contains not less than 1.4 mg and not more than 5.6 mg of saikosaponin b_2, not less than 78 mg and not more than 234 mg of baicalin ($C_{21}H_{18}O_{11}$: 446.36), and not less than 15 mg and

not more than 45 mg of glycyrrhizic acid ($C_{42}H_{62}O_{16}$: 822.93), per extract prepared with the amount specified in the Method of preparation.

Method of preparation

	1)	2)
Bupleurum Root	6 g	6 g
Cinnamon Bark	3 g	3 g
Scutellaria Root	3 g	3 g
Oyster Shell	3 g	3 g
Processed Ginger	2 g	3 g
Glycyrrhiza	2 g	2 g
Trichosanthes Root	3 g	4 g

Prepare a dry extract or viscous extract as directed under Extracts, according to the prescription 1) or 2), using the crude drugs shown above.

Description The dry extract: Saikokeishikankyoto Extract is a light yellow-brown to brown powder. It has a characteristic odor and a hot, bitter and slightly sweet taste.

The viscous extract: Saikokeishikankyoto Extract is a black-brown viscous liquid. It has a characteristic odor and a bitter, hot and slightly sweet taste, followed by an astringent aftertaste.

Identification **(1)** Shake 1.0 g of the dry extract (or 3.0 g of the viscous extract) with 10 mL of water, add 10 mL of 1-butanol, and shake. Centrifuge this solution, and use the 1-butanol layer as the sample solution. Separately, dissolve 1 mg of saikosaponin b_2 for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 5 μL of the sample solution and 2 μL of the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, ethanol (99.5) and water (8:2:1) to a distance of about 7 cm, and air-dry the plate. Spray evenly 4-dimethylaminobenzaldehyde TS for spraying on the plate, and heat the plate at 105°C for 5 minutes. Examine under ultraviolet light (main wavelength: 365 nm): one of the several spots obtained from the sample solution has the same color tone and *R*f value with the yellow fluorescent spot from the standard solution (Bupleurum Root).

(2) Perform the test according to the following i) or ii) (Cinnamon Bark).

i) Put 10 g of dry extract (or 30 g of the viscous extract) in a 300-mL of hard-glass flask, add 100 mL of water and 1 mL of silicone resin, connect the apparatus for essential oil determination, and heat to boil under a reflux condenser. The graduated tube of the apparatus is to be previously filled with water to the standard line, and 2 mL of hexane is added to the graduated tube. After heating under reflux for 1 hour, separate the hexane layer, and use this solution as the sample solution. Separately, dissolve 1 mg of (*E*)-cinnamaldehyde for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 20 μL of the sample solution and 2 μL of the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of hexane, diethyl ether and methanol (15:5:1) to a distance of about 7 cm, and air-dry the plate. Spray evenly 2,4-dinitrophenylhydrazine TS on the plate: one of the several spots obtained from the sample solution has the same color tone and *R*f value with the yellow-orange to orange spot from the standard solution.

ii) Shake 2.0 g of dry extract (or 6.0 g of the viscous extract) with 10 mL of water, add 5 mL of hexane, and shake. Centrifuge this solution, and use the hexane layer as the sample solution. Separately, dissolve 1 mg of (*E*)-2-methoxycinnamaldehyde for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 20 μL of the sample solution and 2 μL of the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of hexane and ethyl acetate (2:1) to a distance of about 7 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 365 nm): one of the several spots obtained from the sample solution has the same color tone and *R*f value with the blue-white fluorescent spot from the standard solution.

(3) Shake 1.0 g of the dry extract (or 3.0 g of the viscous extract) with 10 mL of water, add 25 mL of diethyl ether, and shake. Separate the diethyl ether layer, evaporate the solvent under low pressure (in vacuo), add 2 mL of diethyl ether to the residue, and use this solution as the sample solution. Separately, dissolve 1 mg of wogonin for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 10 μL of the sample solution and 2 μL of the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of hexane and acetone (7:5) to a distance of about 7 cm, and air-dry the plate. Spray evenly iron (III) chloride-methanol TS on the plate: one of the several spots obtained from the sample solution has the same color tone and *R*f value with the yellow-brown to grayish brown spot from the standard solution (Scutellaria Root).

(4) Shake 1.0 g of the dry extract (or 3.0 g of the viscous extract) with 10 mL of water, add 25 mL of diethyl ether, and shake. Separate the diethyl ether layer, evaporate the solvent under low pressure (in vacuo), add 2 mL of diethyl ether to the residue, and use this solution as the sample solution. Separately, dissolve 1 mg of [6]-shogaol for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 20 μL of the sample solution and 5 μL of the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate and hexane (1:1) to a distance of about 7 cm, and air-dry the plate. Spray evenly 4-dimethylaminobenzaldehyde TS for spraying on the plate, heat the plate at 105°C for 5 minutes, allow to cool, and spray water: one of the several

spots obtained from the sample solution has the same color tone and *R*f value with the blue-green to grayish green spot from the standard solution (Processed Ginger).

(5) Shake 1.0 g of the dry extract (or 3.0 g of the viscous extract) with 10 mL of water, add 10 mL of 1-butanol, and shake. Centrifuge this solution, and use the 1-butanol layer as the sample solution. Separately, dissolve 1 mg of liquiritin for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 1 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, methanol and water (20:3:2) to a distance of about 7 cm, and air-dry the plate. Spray evenly dilute sulfuric acid on the plate, heat the plate at 105°C for 5 minutes, and examine under ultraviolet light (main wavelength: 365 nm): one of the several spots obtained from the sample solution has the same color tone and *R*f value with the yellow to yellow-green fluorescent spot from the standard solution (Glycyrrhiza).

Purity **(1)** Heavy metals <*1.07*>—Prepare the test solution with 1.0 g of the dry extract (or an amount of the viscous extract, equivalent to 1.0 g of the dried substance) as directed under the Extracts (4), and perform the test (not more than 30 ppm).

(2) Arsenic <*1.11*>—Prepare the test solution with 0.67 g of the dry extract (or an amount of the viscous extract, equivalent to 0.67 g of the dried substance) according to Method 3, and perform the test (not more than 3 ppm).

Loss on drying <*2.41*> The dry extract: Not more than 9.5% (1 g, 105°C, 5 hours).

The viscous extract: Not more than 66.7% (1 g, 105°C, 5 hours).

Total ash <*5.01*> Not more than 13.0%, calculated on the dried basis.

Assay **(1)** Saikosaponin b_2—Weigh accurately about 0.5 g of the dry extract (or an amount of the viscous extract, equivalent to about 0.5 g of the dried substance), add 20 mL of diethyl ether and 10 mL of water, and shake for 10 minutes. After centrifuging, remove the diethyl ether layer, then add 20 mL of diethyl ether, proceed in the same manner as described above, and remove the diethyl ether layer. To the aqueous layer add 10 mL of methanol, shake for 30 minutes, centrifuge, and separate the supernatant liquid. To the residue add 20 mL of diluted methanol (1 in 2), shake for 5 minutes, centrifuge, and separate the supernatant liquid. Combine all the supernatant liquids, add diluted methanol (1 in 2) to make exactly 50 mL, and use this solution as the sample solution. Separately, use saikosaponin b_2 standard TS for assay as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine the peak areas, A_T and A_S, of saikosaponin b_2 in each solution.

Amount (mg) of saikosaponin $b_2 = C_S \times A_T/A_S \times 50$

C_S: Concentration (mg/mL) of saikosaponin b_2 in saikosaponin b_2 standard TS for assay

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: A mixture of 0.05 mol/L sodium dihydrogen phosphate TS and acetonitrile (5:3).

Flow rate: 1.0 mL per minute.

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of saikosaponin b_2 are not less than 5000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of saikosaponin b_2 is not more than 1.5%.

(2) Baicalin—Weigh accurately about 0.1 g of the dry extract (or an amount of the viscous extract, equivalent to about 0.1 g of the dried substance), add exactly 50 mL of diluted methanol (7 in 10), shake for 15 minutes, filter, and use the filtrate as the sample solution. Separately, weigh accurately about 10 mg of Baicalin RS (separately determine the water <*2.48*> by coulometric titration, using 10 mg), and dissolve in methanol to make exactly 100 mL. Pipet 5 mL of this solution, add diluted methanol (7 in 10) to make exactly 10 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine the peak areas, A_T and A_S, of baicalin in each solution.

Amount (mg) of baicalin ($C_{21}H_{18}O_{11}$)

$= M_S \times A_T/A_S \times 1/4$

M_S: Amount (mg) of Baicalin RS taken, calculated on the anhydrous basis

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 277 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: A mixture of diluted phosphoric acid (1 in 200) and acetonitrile (19:6).

Flow rate: 1.0 mL per minute.

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of baicalin are not less than 5000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of baicalin is not more than 1.5%.

(3) Glycyrrhizic acid—Perform the test according to the following i) or ii).

i) Weigh accurately about 0.5 g of the dry extract (or an amount of the viscous extract, equivalent to about 0.5 g of the dried substance), add exactly 50 mL of diluted methanol (1 in 2), shake for 15 minutes, filter, and use the filtrate as the sample solution. Separately, weigh accurately about 10 mg of Glycyrrhizic Acid RS (separately determine the water <*2.48*> by coulometric titration, using 10 mg), dissolve in diluted methanol (1 in 2) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine the peak areas, A_T and A_S, of glycyrrhizic acid in each solution.

$$\text{Amount (mg) of glycyrrhizic acid } (C_{42}H_{62}O_{16}) = M_S \times A_T/A_S \times 1/2$$

M_S: Amount (mg) of Glycyrrhizic Acid RS taken, calculated on the anhydrous basis

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: Dissolve 3.85 g of ammonium acetate in 720 mL of water, and add 5 mL of acetic acid (100) and 280 mL of acetonitrile.

Flow rate: 1.0 mL per minute.

System suitability—

System performance: Dissolve 5 mg of monoammonium glycyrrhizinate for resolution check in 20 mL of dilute ethanol. When the procedure is run with 10 μL of this solution under the above operating conditions, the resolution between the peak having the relative retention time of about 0.9 to glycyrrhizic acid and the peak of glycyrrhizic acid is not less than 1.5. Dissolve 1 mg of (*E*)-cinnamaldehyde for thin-layer chromatography and 1 mg of baicalein for resolution check in 50 mL of methanol. To 2 mL of this solution add 2 mL of the standard solution. When the procedure is run with 10 μL of this solution under the above operating conditions, two peaks other than glycyrrhizic acid are observed with the resolutions between the peak of glycyrrhizic acid and each of the two peaks being not less than 1.5.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of glycyrrhizic acid is not more than 1.5%.

ii) Weigh accurately about 0.5 g of the dry extract (or an amount of the viscous extract, equivalent to about 0.5 g of the dried substance), add 20 mL of diethyl ether and 10 mL of water, and shake for 10 minutes. After centrifuge, remove the diethyl ether layer, add 20 mL of diethyl ether, proceed in the same manner as described above, and remove the diethyl ether layer. To the aqueous layer add 10 mL of methanol, shake for 30 minutes, centrifuge, and separate the supernatant liquid. To the residue add 20 mL of diluted methanol (1 in 2), shake for 5 minutes, centrifuge, and take the supernatant liquid. Combine these supernatant liquids, add diluted methanol (1 in 2) to make exactly 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 10 mg of Glycyrrhizic Acid RS (separately determine the water <*2.48*> by coulometric titration, using 10 mg), dissolve in diluted methanol (1 in 2) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine the peak areas, A_T and A_S, of glycyrrhizic acid in each solution.

$$\text{Amount (mg) of glycyrrhizic acid } (C_{42}H_{62}O_{16}) = M_S \times A_T/A_S \times 1/2$$

M_S: Amount (mg) of Glycyrrhizic Acid RS taken, calculated on the anhydrous basis

Operating conditions—

Proceed as directed in the operating conditions in i).

System suitability—

System repeatability: Proceed as directed in the system suitability in i).

System performance: Dissolve 5 mg of monoammonium glycyrrhizinate for resolution check in 20 mL of dilute ethanol. When the procedure is run with 10 μL of this solution under the above operating conditions, the resolution between the peak having the relative retention time of about 0.9 to glycyrrhizic acid and the peak of glycyrrhizic acid is not less than 1.5.

Containers and storage Containers—Tight containers.

Senna Leaf

センナ

Change the Description and Identification (2) as follows (No effect to English text for Description):

Identification

(2) To 2 g of pulverized Senna Leaf add 20 mL of a mixture of tetrahydrofuran, methanol and dilute hydrochloric acid (16:4:1), shake for 5 minutes, filter, and use the filtrate as the sample solution. Separately, dissolve 1 mg of Sennoside A RS or sennoside A for thin-layer chromatography in 1 mL of a mixture of tetrahydrofuran and water (7:3), and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of 1-propanol, ethyl acetate, water and acetic acid (100) (40:40:30:1) to a distance of about 7 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 365 nm): one of the several spots obtained from the sample solution has the same color tone and *R*f value with the red to dark red fluorescent spot from the standard solution.

Powdered Senna Leaf

センナ末

Change the Identification (2) as follows:

Identification

(2) To 2 g of Powdered Senna Leaf add 20 mL of a mixture of tetrahydrofuran, methanol and dilute hydrochloric acid (16:4:1), shake for 5 minutes, filter, and use the filtrate as the sample solution. Separately, dissolve 1 mg of Sennoside A RS or sennoside A for thin-layer chromatography in 1 mL of a mixture of tetrahydrofuran and water (7:3), and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 5 μL each of the sample solution and standard solutions on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of 1-propanol, ethyl acetate, water and acetic acid (100) (40:40:30:1) to a distance of about 7 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 365 nm): one of the several spots obtained from the sample solution has the same color tone and *R*f value with the red to dark red fluorescent spot from the standard solution.

Shimbuto Extract

真武湯エキス

Change the Assay (2) as follows:

Assay

(2) [6]-gingerol—Weigh accurately about 0.5 g of Shimbuto Extract, add exactly 50 mL of diluted methanol (7 in 10), shake for 15 minutes, filter, and use the filtrate as the sample solution. Separately, weigh accurately about 10 mg of [6]-gingerol for assay, dissolve in methanol to make exactly 100 mL. Pipet 5 mL of this solution, add methanol to make exactly 50 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine the peak areas, A_T and A_S, of [6]-gingerol in each solution.

$$\text{Amount (mg) of [6]-gingerol} = M_S \times A_T/A_S \times 1/20$$

M_S: Amount (mg) of [6]-gingerol for assay taken, calculated on the basis of the content obtained by qNMR

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 282 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 30°C.

Mobile phase: A mixture of water, acetonitrile and phosphoric acid (620:380:1).

Flow rate: 1.0 mL per minute (the retention time of [6]-gingerol is about 15 minutes).

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of [6]-gingerol are not less than 5000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of [6]-gingerol is not more than 1.5%.

Sinomenium Stem and Rhizome

ボウイ

Change the origin/limits of content as follows:

Sinomenium Stem and Rhizome is the climbing stem and rhizome of *Sinomenium acutum* Rehder et E. H. Wilson (*Menispermaceae*), usually cut transver-

sely.

Tokakujokito Extract

桃核承気湯エキス

Change the Assay (1) as follows:

Assay

(1) Amygdalin—Weigh accurately about 0.5 g of Tokakujokito Extract, add exactly 50 mL of diluted methanol (1 in 2), shake for 15 minutes, and filter. Pipet 5 mL of the filtrate, elute through a column prepared previously with 2 g of polyamide for column chromatography using water to make exactly 20 mL of effluent , and use this solution as the sample solution. Separately, weigh accurately about 10 mg of amygdalin for assay, dissolve in diluted methanol (1 in 2) to make exactly 50 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine the peak areas, A_T and A_S, of amygdalin in each solution.

$$\text{Amount (mg) of amygdalin} = M_S \times A_T/A_S \times 4$$

M_S: Amount (mg) of amygdalin for assay taken

Operation conditions—

Detector: An ultraviolet absorption photometer (wavelength: 210 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 45°C.

Mobile phase: A mixture of 0.05 mol/L sodium dihydrogen phosphate TS and methanol (5:1).

Flow rate: 0.8 mL per minute (the retention time of amygdalin is about 12 minutes).

Systemic suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of amygdalin are not less than 5000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of amygdalin is not more than 1.5%.

Turmeric

ウコン

Change the Description as follows (No effect to English text):

Uncaria Hook

チョウトウコウ

Change the Assay as follows:

Assay Weigh accurately about 0.2 g of moderately fine powder of Uncaria Hook, transfer into a glass-stoppered centrifuge tube, add 30 mL of a mixture of methanol and dilute acetic acid (7:3), shake for 30 minutes, centrifuge, and separate the supernatant liquid. To the residue add two 10-mL portions of a mixture of methanol and dilute acetic acid (7:3), proceed in the same manner, and combine all of the supernatant liquid. To the combined liquid add a mixture of methanol and dilute acetic acid (7:3) to make exactly 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 5 mg of rhynchophylline for assay, and dissolve in a mixture of methanol and dilute acetic acid (7:3) to make exactly 100 mL. Pipet 1 mL of this solution, add a mixture of methanol and dilute acetic acid (7:3) to make exactly 10 mL, and use this solution as the standard solution (1). Separately, dissolve 1 mg of hirsutine in 100 mL of a mixture of methanol and dilute acetic acid (7:3), and use this solution as the standard solution (2). Perform the test with exactly 20 μL each of the sample solution and standard solutions (1) and (2) as directed under Liquid Chromatography <*2.01*> according to the following conditions. Determine the peak areas, A_{Ta} and A_{Tb}, of rhynchophylline and hirsutine obtained from the sample solution, and the peak area, A_S, of rhynchophylline from the standard solution (1).

$$\text{Amount (mg) of total alkaloids (rhynchophylline and hirsutine)} = M_S \times (A_{Ta} + 1.405A_{Tb})/A_S \times 1/20$$

M_S: Amount (mg) of rhynchophylline for assay taken

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 245 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: Dissolve 3.85 g of ammonium acetate in 200 mL of water, add 10 mL of acetic acid (100) and water to make 1000 mL, and add 350 mL of acetonitrile.

Flow rate: Adjust so that the retention time of rhyn-

chophylline is about 17 minutes.

System suitability—

System performance: Dissolve 5 mg of rhynchophylline for assay in 100 mL of a mixture of methanol and dilute acetic acid (7:3). To 5 mL of this solution add 1 mL of ammonia solution (28), and warm at about 50°C for 2 hours or heat under a reflux condenser for 10 minutes. After cooling, to 1 mL of the solution so obtained add a mixture of methanol and dilute acetic acid (7:3) to make 5 mL. When the procedure is run with 20 μL of this solution under the above operating conditions, the peak of isorhynchophylline appears in addition to the peak of rhynchophylline, and the resolution between these peaks is not less than 1.5.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution (1) under the above operating conditions, the relative standard deviation of the peak areas of rhynchophylline is not more than 1.5%.

Add the following:

Yokukansankachimpihange Extract

抑肝散加陳皮半夏エキス

Yokukansankachimpihange Extract contains not less than 0.6 mg and not more than 2.4 mg of saikosaponin b_2, not less than 10 mg and not more than 30 mg of glycyrrhizic acid ($C_{42}H_{62}O_{16}$: 822.93), and not less than 18 mg and not more than 72 mg of hesperidin, per extract prepared with the amount specified in the Method of preparation.

Method of preparation

	1)	2)
Japanese Angelica Root	3 g	3 g
Uncaria Hook	3 g	3 g
Cnidium Rhizome	3 g	3 g
Atractylodes Rhizome	4 g	—
Atractylodes Lancea Rhizome	—	4 g
Poria Sclerotium	4 g	4 g
Bupleurum Root	2 g	2 g
Glycyrrhiza	1.5 g	1.5 g
Citrus Unshiu Peel	3 g	3 g
Pinellia Tuber	5 g	5 g

Prepare a dry extract or viscous extract as directed under Extracts, according to the prescription 1) or 2), using the crude drugs shown above.

Description The dry extract: Yokukansankachimpihange Extract is a grayish brown to reddish yellow-brown powder. It has a characteristic odor, and has a sweet and slightly hot taste at first, later bitter.

The viscous extract: Yokukansankachimpihange Extract is a brown viscous liquid. It has a characteristic odor, and has a bitter and slightly sweet taste.

Identification **(1)** Shake 2.0 g of the dry extract (or 6.0 g of the viscous extract) with 10 mL of water, then add 10 mL of diethyl ether, shake, and centrifuge. Separate the diethyl ether layer, add 10 mL of sodium hydroxide TS, and shake. Centrifuge this solution, and use the diethyl ether layer as the sample solution. Separately, use (*Z*)-ligustilide TS for thin-layer chromatography as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of butyl acetate and hexane (2:1) to a distance of about 7 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 365 nm): one of the several spots obtained from the sample solution has the same color tone and *R*f value with the blue-white fluorescent spot from the standard solution (Japanese Angelica Root; Cnidium Rhizome).

(2) Shake 2.0 g of the dry extract (or 6.0 g of the viscous extract) with 20 mL of water and 2 mL of ammonia TS, add 20 mL of diethyl ether, and shake. Separate the diethyl ether layer, evaporate the solvent under low pressure (in vacuo), add 1 mL of methanol to the residue, and use this solution as the sample solution. Separately, dissolve 1 mg each of rhyncophyllin for thin-layer chromatography and hirsutine for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 10 μL of the sample solution and 2 μL of the standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, 1-propanol, water and acetic acid (100) (7:5:4:1) to a distance of about 7 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): at least one of the several spots obtained from the sample solution has the same color tone and *R*f value with one of the two dark violet spots from the standard solution (Uncaria Hook).

(3) For preparation prescribed Atractylodes Rhizome— Shake 1.0 g of the dry extract (or 3.0 g of the viscous extract) with 10 mL of water, add 25 mL of diethyl ether, and shake. Separate the diethyl ether layer, evaporate the solvent under low pressure (in vacuo), add 2 mL of diethyl ether to the residue, and use this solution as the sample solution. Separately, dissolve 1 mg of atractylenoide III for thin-layer chromatography in 2 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of hexane and ethyl acetate (2:1) to a distance of about 7 cm, and air-dry the plate. Spray evenly 1-naphthol-sulfuric acid TS on the plate, heat the plate at 105°C for 5 minutes, and allow to cool: one of the several spots obtained from the sample solution has the same color tone and *R*f value with the red to red-purple spot from the standard solution (Atractylodes Rhizome).

(4) For preparation prescribed Atractylodes Lancea

Rhizome—Shake 2.0 g of the dry extract (or 6.0 g of the viscous extract) with 10 mL of water, add 25 mL of hexane, and shake. Separate the hexane layer, evaporate the solvent under low pressure (in vacuo), add 2 mL of hexane to the residue, and use this solution as the sample solution. Perform the test with the sample solution as directed under Thin-layer Chromatography <*2.03*>. Spot 20 μL of the sample solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of hexane and acetone (7:1) to a distance of about 7 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): a dark purple spot is observed at an *R*f value of about 0.5. The spot shows a green-brown color after being sprayed evenly 4-dimethylaminobenzaldehyde TS for spraying, heated at 105°C for 5 minutes, and allowed to cool (Atractylodes Lancea Rhizome).

(5) Shake 1.0 g of the dry extract (or 3.0 g of the viscous extract) with 10 mL of water, add 10 mL of 1-butanol, and shake. Centrifuge this solution, and use the 1-butanol layer as the sample solution. Separately, dissolve 1 mg of saikosaponin b_2 for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 10 μL of the sample solution and 2 μL of the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, ethanol (99.5) and water (8:2:1) to a distance of about 7 cm, and air-dry the plate. Spray evenly 4-dimethylaminobenzaldehyde TS for spraying on the plate, heat the plate at 105°C for 5 minutes, and examine under ultraviolet light (main wavelength: 365 nm): one of the several spots obtained from the sample solution has the same color tone and *R*f value with the yellow fluorescent spot from the standard solution (Bupleurum Root).

(6) Shake 1.0 g of the dry extract (or 3.0 g of the viscous extract) with 10 mL of water, add 10 mL of 1-butanol, and shake. Centrifuge this solution, and use the 1-butanol layer as the sample solution. Separately, dissolve 1 mg of liquiritin for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 1 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, methanol and water (20:3:2) to a distance of about 7 cm, and air-dry the plate. Spray evenly dilute sulfuric acid on the plate, heat the plate at 105°C for 5 minutes, and examine under ultraviolet light (main wavelength: 365 nm): one of the several spots obtained from the sample solution has the same color tone and *R*f value with the yellow-green fluorescent spot from the standard solution (Glycyrrhiza).

(7) Shake 1.0 g of the dry extract (or 3.0 g of the viscous extract) with 10 mL of water, add 10 mL of 1-butanol, centrifuge, and use the 1-butanol layer as the sample solution. Separately, dissolve 1 mg of hesperidin for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 20 μL of the sample solution and 10 μL of the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, acetone, water and acetic acid (100) (10:6:3:1) to a distance of about 7 cm, and air-dry the plate. Spray evenly 2,6-dibromo-*N*-chloro-1,4-benzoquinone monoimine TS on the plate, and expose the plate to ammonia vapor: one of the several spots from the sample solution has the same color tone and *R*f value with the blue spot from the standard solution (Citrus Unshiu Peel).

Purity **(1)** Heavy metals <*1.07*>—Prepare the test solution with 1.0 g of the dry extract (or an amount of the viscous extract, equivalent to 1.0 g of the dried substance) as directed under Extracts (4), and perform the test (not more than 30 ppm).

(2) Arsenic <*1.11*>—Prepare the test solution with 0.67 g of the dry extract (or an amount of the viscous extract, equivalent to 0.67 g of the dried substance) according to Method 3, and perform the test (not more than 3 ppm).

Loss on drying <*2.41*> The dry extract: Not more than 10.0% (1 g, 105°C, 5 hours).

The viscous extract: Not more than 66.7% (1 g, 105°C, 5 hours).

Total ash <*5.01*> Not more than 9.0%, calculated on the dried basis.

Assay **(1)** Saikosaponin b_2—Weigh accurately about 0.5 g of the dry extract (or an amount of the viscous extract, equivalent to about 0.5 g of the dried substance), add 20 mL of diethyl ether and 10 mL of water, and shake for 10 minutes. After centrifugation, remove the diethyl ether layer, then add 20 mL of diethyl ether, proceed in the same manner as described above, and remove the diethyl ether layer. To the aqueous layer add 10 mL of methanol, shake for 30 minutes, centrifuge, and separate the supernatant liquid. To the residue add 20 mL of diluted methanol (1 in 2), shake for 5 minutes, then centrifuge, and separate the supernatant liquid. Combine all the supernatant liquids, add diluted methanol (1 in 2) to make exactly 50 mL, and use this solution as the sample solution. Separately, use saikosaponin b_2 standard TS for assay as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine the peak areas, A_T and A_S, of saikosaponin b_2 in each solution.

$$\text{Amount (mg) of saikosaponin } b_2 = C_S \times A_T/A_S \times 50$$

C_S: Concentration (mg/mL) of saikosaponin b_2 in saikosaponin b_2 standard TS for assay

Operation conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diame-

ter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: A mixture of 0.05 mol/L sodium dihydrogen phosphate TS and acetonitrile (5:3).

Flow rate: 1.0 mL per minute.

Systemic suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of saikosaponin b_2 are not less than 5000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of saikosaponin b_2 is not more than 1.5%.

(2) Glycyrrhizic acid—Weigh accurately about 0.5 g of the dry extract (or an amount of the viscous extract, equivalent to about 0.5 g of the dried substance), add 20 mL of diethyl ether and 10 mL of water, and shake for 10 minutes. After centrifugation, remove the diethyl ether layer, then add 20 mL of diethyl ether, proceed in the same manner as above, and remove the diethyl ether layer. To the aqueous layer add 10 mL of methanol, shake for 30 minutes, then centrifuge, and separate the supernatant liquid. To the residue add 20 mL of diluted methanol (1 in 2), shake for 5 minutes, centrifuge, and take the supernatant liquid. Combine the supernatant liquids, add diluted methanol (1 in 2) to make exactly 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 10 mg of Glycyrrhizic Acid RS (separately determine the water <2.48> by coulometric titration, using 10 mg), dissolve in diluted methanol (1 in 2) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the peak areas, A_T and A_S, of glycyrrhizic acid in each solution.

$$\text{Amount (mg) of glycyrrhizic acid } (C_{42}H_{62}O_{16}) = M_S \times A_T/A_S \times 1/2$$

M_S: Amount (mg) of Glycyrrhizic Acid RS taken, calculated on the anhydrous basis

Operation conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: Dissolve 3.85 g of ammonium acetate in 720 mL of water, and add 5 mL of acetic acid (100) and 280 mL of acetonitrile.

Flow rate: 1.0 mL per minute.

Systemic suitability—

System performance: Dissolve 5 mg of monoammonium glycyrrhizinate for resolution check in 20 mL of dilute ethanol. When the procedure is run with 10 μL of this solution under the above operating conditions, the resolution between the peak having the relative retention time about 0.9 to glycyrrhizic acid and the peak of glycyrrhizic acid is not less than 1.5.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of glycyrrhizic acid is not more than 1.5%.

(3) Hesperidin—Weigh accurately about 0.1 g of the dry extract (or an amount of the viscous extract, equivalent to about 0.1 g of dried substance), add exactly 50 mL of diluted tetrahydrofuran (1 in 4), shake for 30 minutes, centrifuge, and use the supernatant liquid as the sample solution. Separately, weigh accurately about 10 mg of hesperidin for assay, previously dried in a desiccator (silica gel) for 24 hours, dissolve in methanol to make exactly 100 mL. Pipet 10 mL of this solution, add diluted tetrahydrofuran (1 in 4) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the peak areas, A_T and A_S, of hesperidin in each solution.

$$\text{Amount (mg) of hesperidin} = M_S \times A_T/A_S \times 1/20$$

M_S: Amount (mg) of hesperidin for assay taken

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 285 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: A mixture of water, acetonitrile and acetic acid (100) (82:18:1).

Flow rate: 1.0 mL per minute.

System suitability—

System performance: Dissolve 1 mg each of hesperidin for assay and naringin for thin-layer chromatography in diluted methanol (1 in 2) to make 100 mL. When the procedure is run with 10 μL of this solution under the above operating conditions, naringin and hespeidin are eluted in this order with the resolution between these peaks being not less than 1.5.

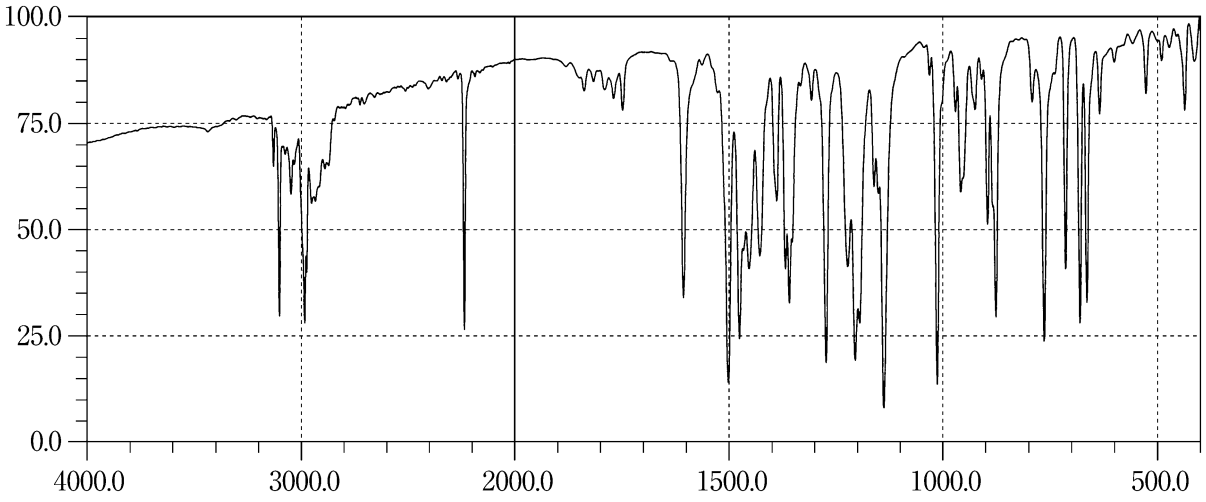
System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of hesperidin is not more than 1.5%.

Containers and storage Containers—Tight containers.

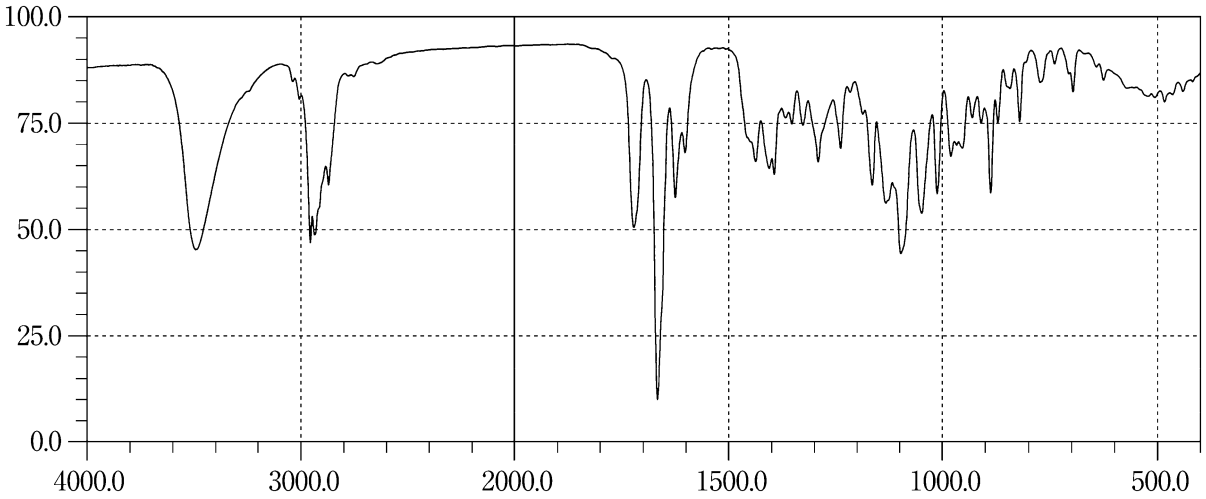
Infrared
Reference Spectra

Add the following spectra:

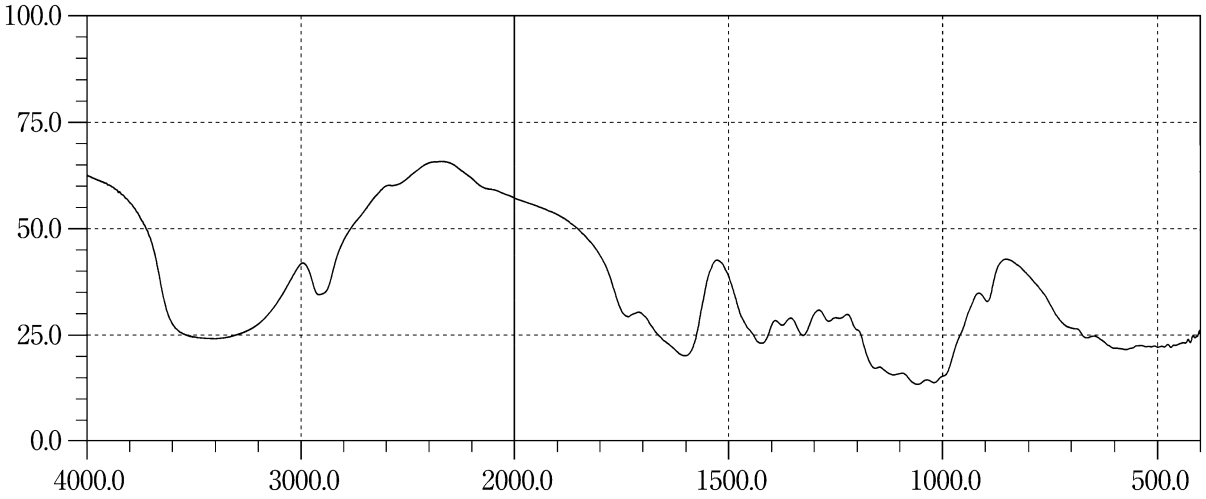
Anastrozole



Budesonide

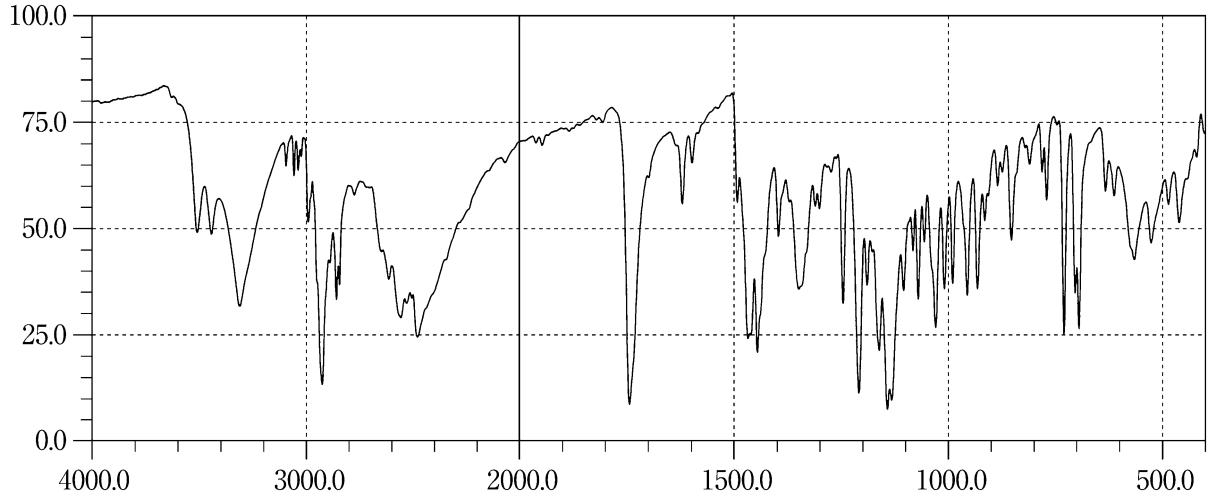


Croscarmellose Sodium

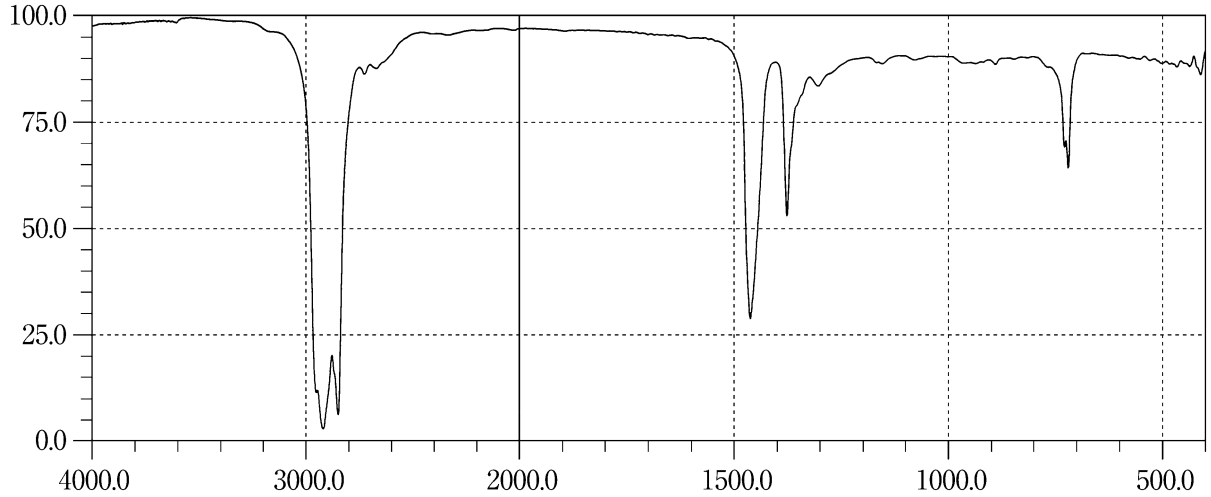


The JP Drugs are to be tested according to the provisions given in the pertinent monographs, General Notices, General Rules for Crude Drugs, General Rules for Preparations, and General Tests for their conformity to the Japanese Pharmacopoeia. (*See the General Notices 5.*)

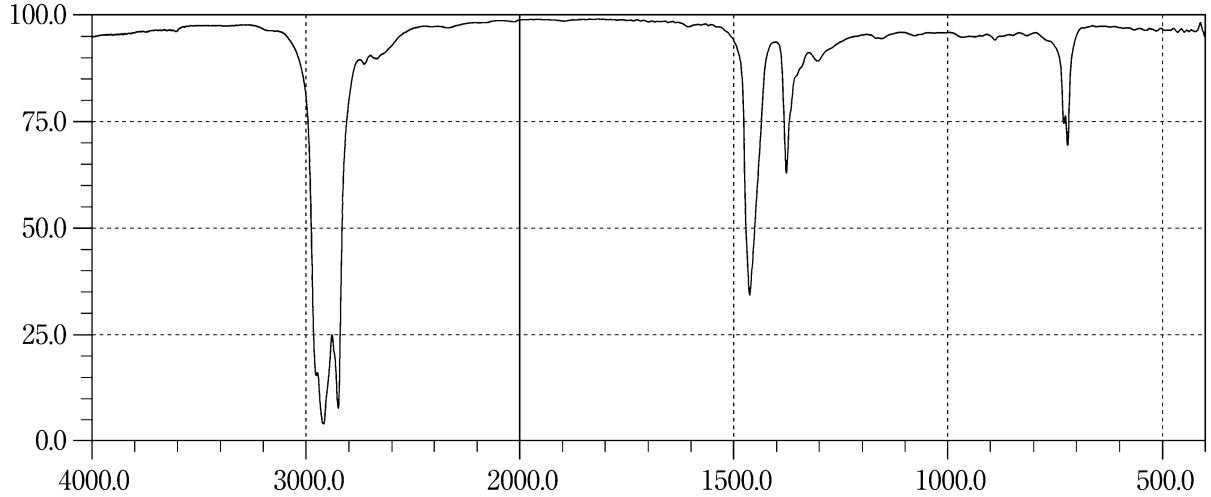
Oxybutynin Hydrochloride



White Petrolatum

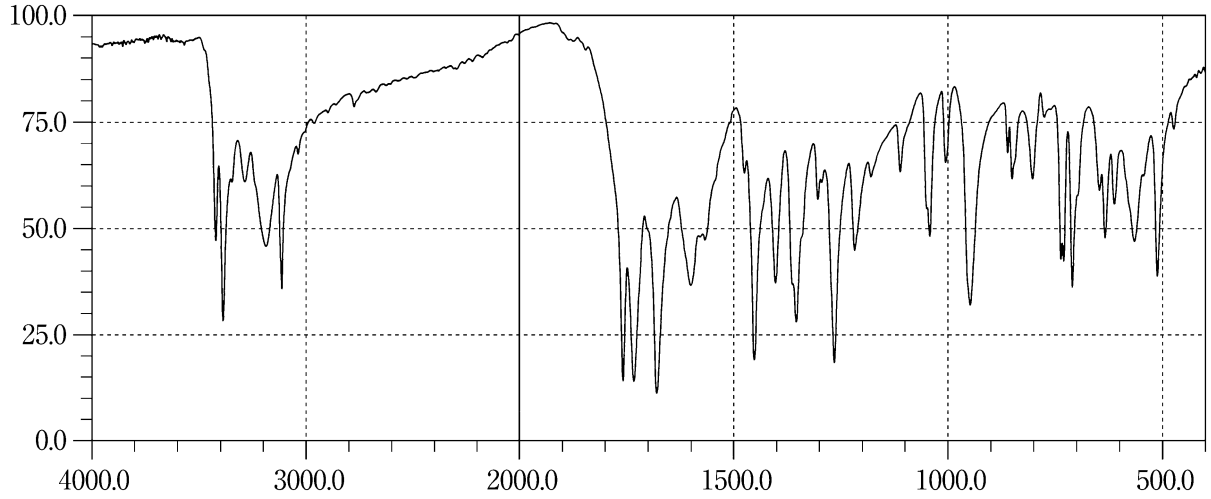


Yellow Petrolatum



The JP Drugs are to be tested according to the provisions given in the pertinent monographs, General Notices, General Rules for Crude Drugs, General Rules for Preparations, and General Tests for their conformity to the Japanese Pharmacopoeia. (*See the General Notices 5.*)

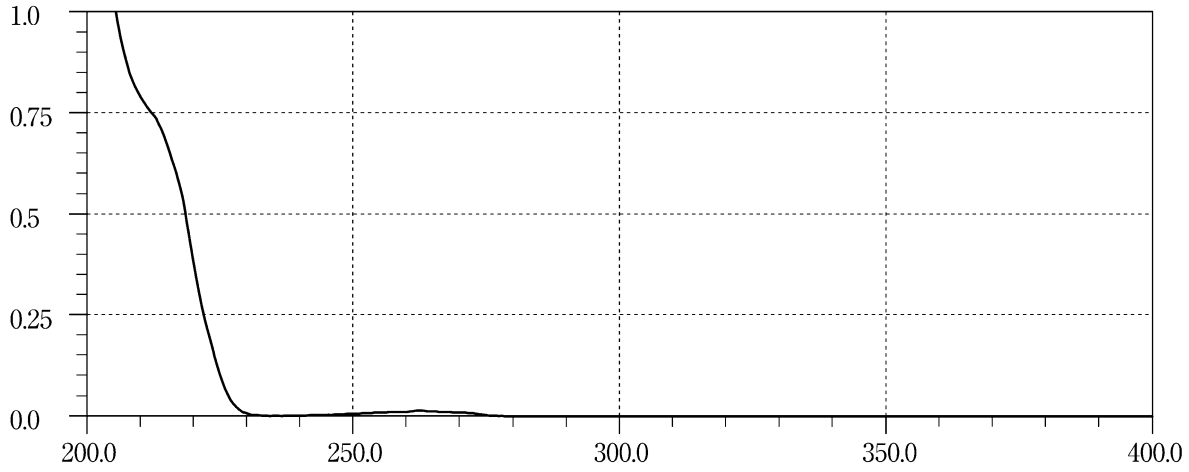
Temozolomide



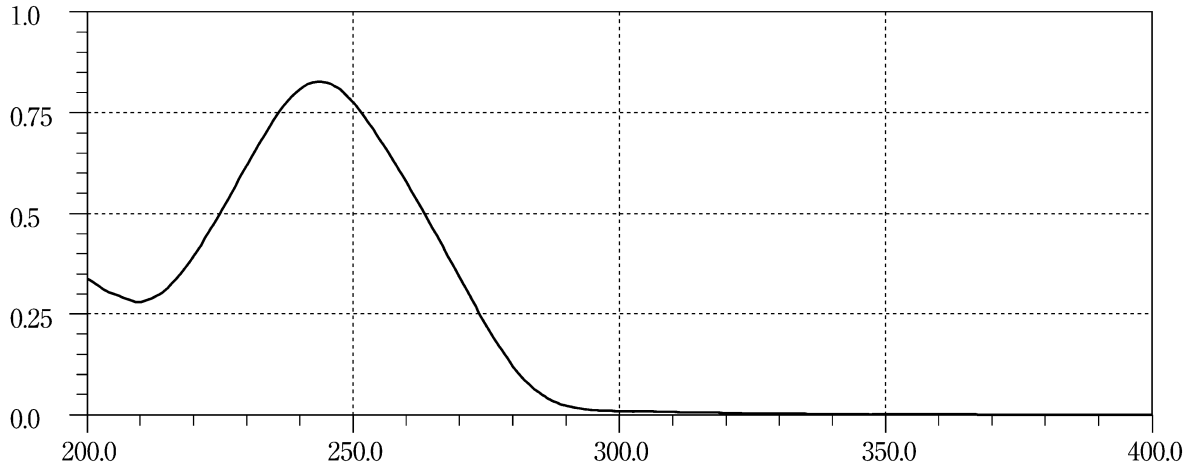
Ultraviolet-visible Reference Spectra

Add the following spectra:

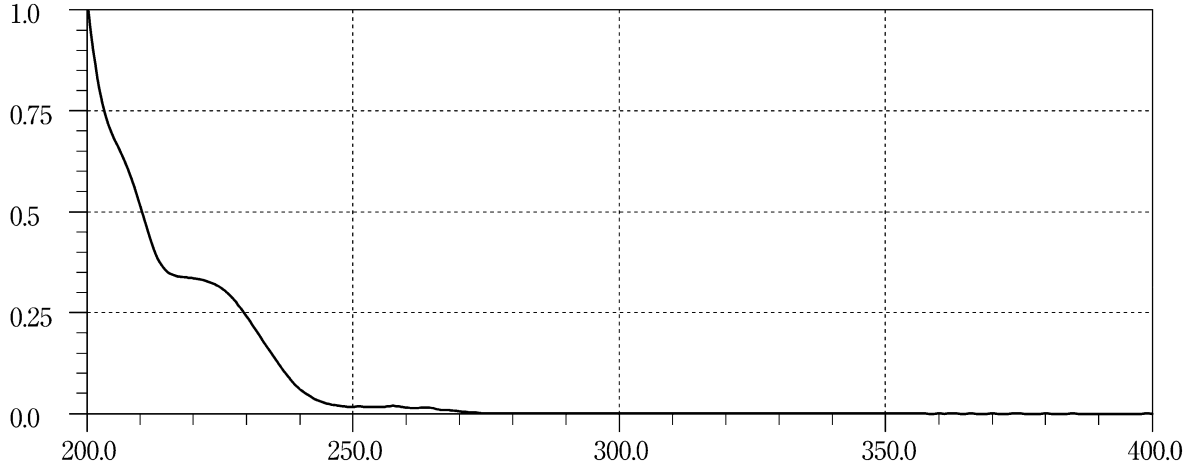
Anastrozole



Budesonide

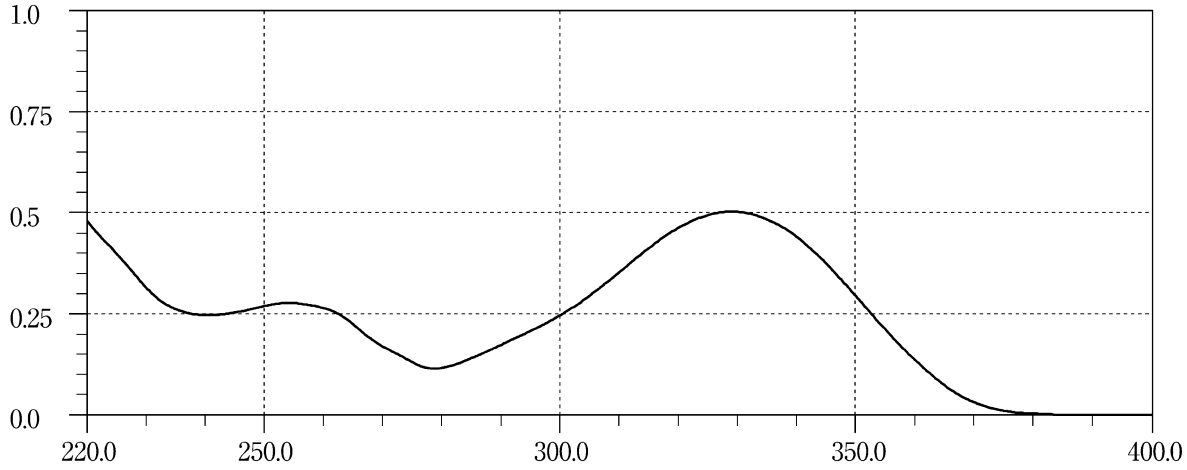


Oxybutynin Hydrochloride



The JP Drugs are to be tested according to the provisions given in the pertinent monographs, General Notices, General Rules for Crude Drugs, General Rules for Preparations, and General Tests for their conformity to the Japanese Pharmacopoeia. (*See the General Notices 5.*)

Temozolomide



General Information

GENERAL INFORMATION

The General Information describes reference information and reference test methods necessary to assure the quality of medicines, which is attached to the JP. Therefore, the General Information is positioned as important information supplementing the JP although it should not be taken as indicating standards for conformity of drugs, except in the case specified when the drugs are granted approval based on the Law on Securing Quality, Efficacy and Safety of Products Including Pharmaceuticals and Medical Devices. Combination use of the General Information and the JP can contribute to improve quality of the JP and user's convenience.

The general information is classified into the following categories according to their contents, and each general information is individually numbered.

An individual number consists of three blocks. The left block indicates the category number and the central block indicates the number in the category. The figures in right block consist of the first two digits from the left indicating the JP at the recent revision (or new preparation, if not revised) and the third digit indicating as follows: 0 for major revision, 1 for supplement I, 2 for supplement II, and 3 for partial revision. For citation between the general information, the number corresponding to the general information is indicated in angle brackets 〈 〉.

G0. Basic Concepts on Pharmaceutical Quality
G1. Physics and Chemistry
G2. Solid-state Properties
G3. Biotechnological/Biological Products
G4. Microorganisms
G5. Crude Drugs
G6. Drug Formulation
G7. Containers and Package
G8. Reference Standards
GZ. Others

The salient points of the revision in this volume are as follows:

1. ''G9. Pharmaceutical Excipients'' was newly added to the category of the general information.

2. The following were newly prepared.

(1) Instrumental Measurement of Coloration of Liquids *〈G1-4-181〉*

(2) Control Strategies and Change Control Concepts at Each Stage of Chromatography Lifecycle (Change Control in Chromatography Lifecycle) *〈G1-5-181〉*

(3) Measurement of Powder Flow Properties by Shear Cell Methods *〈G2-5-181〉*

(4) Biorisk Management of the Handling of Microorganisms in Microbial Tests *〈G4-11-181〉*

(5) Functionality-related Characteristics of Excipients Relevant to Preparations *〈G9-1-181〉*

3. The following were revised.

(1) Concept on Impurities in Chemically Synthesized Drug Substances and Drug Products *〈G0-3-181〉*

(2) System Suitability *〈G1-2-181〉*

(3) On the Scientific Names of Crude Drugs listed in the JP *〈G5-1-181〉*

(4) Tablet Friability Test *〈G6-5-181〉*

(5) Quality Control of Water for Pharmaceutical Use *〈GZ-2-181〉*

4. The following was deleted.

(1) Near Infrared Spectrometry *〈G1-3-161〉*

G0 Basic Concepts on Pharmaceutical Quality

Concept on Impurities in Chemically Synthesized Drug Substances and Drug Products *〈G0-3-181〉*

Change the following as follows:

1. Classification of impurities found in chemically synthesized pharmaceuticals and the guidance to comply with for their control

Impurities found in chemically synthesized pharmaceuticals are roughly classified into organic impurities, inorganic impurities and residual solvents. Those impurities in new drug substances and products are controlled by the following guidelines agreed upon at the International Council for Harmonization of Technical Requirements for Pharmaceuticals for Human Use (hereinafter referred to as ''ICH''). More specifically, ''Impurities in New Drugs Substances (PAB/PCD Notification No. 877 dated September 25, 1995)'' (hereinafter referred to as ''ICH Q3A Guideline'')[1] on specifications for organic impurities in drug substances applies to applications for marketing approval after April 1, 1997, while ''Impurities in New Drug Products (PAB/PCD Notification No. 539 dated June 23, 1997'' (hereinafter referred to as ''ICH Q3B Guideline'')[2] on specifications for organic impurities in drug products applies to applications for marketing approval after April 1, 1999. Meanwhile, specifications for inorganic impurities were specified by Japanese pharmacopoeial standards and known safety data.

Now ''Guideline for Elemental Impurities (PFSB/ELD Notification No. 4 dated September 30, 2015)'' (hereinafter referred to as ''ICH Q3D Guideline'') applies to applications for marketing approval after April 1, 2017. In regard to residual solvents, ''Impurities: Guideline for Residual Solvents (PAB/ELD Notification No. 307 dated March 30, 1998)'' (hereinafter referred to as ''ICH Q3C Guideline'') applies to applications for marketing approval after April 1, 2000. Especially in regard to DNA-reactive impurities, ''Assessment and control of DNA reactive (mutagenic) impurities in pharmaceuticals to limit potential carcinogenic risk (PSEHB/ELD Notification No. 3 dated November 10, 2015)'' applies to applications for marketing approval after January 15, 2016. Although ICH Q3A guideline does not cover optical enantiomers, a type of organic impurities, ''Specifications: Test Procedures and Acceptance Criteria for New Drug Substances and New Drug Products: Chemical Substances (PMSB/ELD Notification No. 568 dated May 1, 2001)'' (hereinafter referred to as ''ICH Q6A Guideline''), which was published subsequently, provides that enantiomers are impurities that should be controlled and, if measurable, should be controlled in accordance with the principle of the ICH Q3A guideline.

Control of impurities in accordance with the guidelines mentioned above is expected also for pharmaceuticals other than new drug substances and new drug products. Their applications for marketing (or applications for partial changes) are subject to those guidelines when necessary. The General Notices of the JP 17th Edition states that residual solvents of all JP-listed drugs, in principle, have to be controlled in accordance with specification ''Residual Solvents'' in General Tests unless otherwise specified in the individual monograph. In regard to elemental impurities, their tests and control methods have been added stepwise as introduction into the JP. In the JP 18th Edition, the provision regarding elemental impurities based on the ICH Q3D guideline was provided in the Paragraph 34 of General Notices, and also the General Test ''Elemental Impurities ⟨*2.66*⟩'' and General Information ''Control of Elemental Impurities in Drug Products'' were integrated to provide the General Test ''Elemental Impurities ⟨*2.66*⟩'' that reflects the revision of the ICH Q3D guideline.

2. The concept of ICH Q3A and Q3B guidelines for the control of organic impurities

ICH Q3A and Q3B guidelines require setting acceptance criteria for organic impurities based on the information gained from development stages for new drugs. Concerning impurities in drug substances, ICH Q3A guideline refers to the items to be examined from chemical and safety perspectives. ICH Q3B guideline complements Q3A guideline, and has the same basic concept as Q3A. Chemical aspects to be examined include classification and identification of impurities, their reporting method, specification settings and analytical methods. Safety aspects include specific guidelines for qualifying the safety of impurities that were not present, or were present at substantially lower levels, in batches of a drug substance used in safety and clinical studies.

Qualification of the safety is the process of acquiring and evaluating data that establishes the biological safety of an individual impurity or a given impurity profile at the level(s) specified. The applicant should describe a rationale for establishing impurity acceptance criteria that includes safety considerations in attachments when applicated for approval. The level of any impurities present in a new drug substance that has been adequately tested in safety and/or clinical studies would be considered qualified.

Identified impurities, unidentified impurities and total impurities are specified based on the data obtained according to the guidelines. The threshold of unspecified impurities in a drug substance is determined depending on the daily intake of the drug substance. When the maximum daily dosage is not more than 2 g, it is set at 0.10%. The establishment of individual specifications is required for impurities at a level greater than 0.10%.

In regard to drug products, the ICH Q3B guideline cover the degradation products of drug substances or reaction products between the drug substance and additive/primary packaging. Therefore, even if organic impurities other than degradation products (e.g., by-products and synthetic intermediates) in the drug substance are found as impurities in the drug product, they need not be monitored or specified since they have already been controlled as the drug substance specifications. However, degradation products elevated in the drug product need to be monitored and specified.

3. Principles for controlling organic impurities in the articles listed in the JP

Conventionally in the JP, specified impurities, unspecified impurities and total impurities are specified in accordance with ICH Q3A and Q3B guidelines for pharmaceutical products, whose impurities have been controlled by those guidelines, in the process listing in the JP. (However, this shall not apply to the long-term listed pharmaceutical products which had existed in the JP before these guidelines were applicable. However, when a new application is filed for those JP-listed pharmaceutical products, control of impurities in accordance with ICH Q3A and Q3B guidelines may be required, if necessary.) In order to specify the impurities, analysis data during development submitted from the drafting company and impurity analysis data from commercial production batches after consistent manufacturing is achieved should be assessed. Safety evaluation is not required again for the process listing in the JP since it has been performed at the time of approval.

ICH Q3A and Q3B guidelines cover impurities in the drug substances manufactured by chemical syntheses and the drug products manufactured with those drug substances. Similarly, the following types of products are not covered in the JP: biological/biotechnological products, peptides, oligonucleotides, radiopharmaceuticals, fermentation products and semi-synthetic products derived therefrom, herbal products and crude products of animal or plant origin.

When organic impurities assessed in accordance with the

principles of ICH Q3A and Q3B guidelines are listed as JP tests of purity, the operational rationality of the JP is considered and its own modification is added. (i) Except in exceptional circumstances, impurity reference standards are not established. In order to identify an impurity using liquid chromatography, the relative retention time of the impurity to the drug substance is used for identification. (ii) When only unidentified impurities in highly pure pharmaceutical products (not more than 0.1%) are specified, it is generally exempted to set acceptance criteria for total impurities. (iii) When acceptance criteria set based only on actual measured values result in many impurities with slightly different acceptance criteria, consideration can be given so that the purity test consists of a small number of representative acceptance criteria, if possible. (iv) Chemical structural information and the chemical name of the impurities are not disclosed. Those measures enable impurity control without impurity reference standards, and can simplify system suitability tests for highly pure pharmaceutical products.

Meanwhile, the method to identify impurities by use of relative retention time is column-dependent and analysis becomes difficult when appropriate columns are not available. Therefore, the JP 17th Edition also allows the use of the analysis method with impurity reference standards when designing purity tests for a drug substance. In addition, the JP adopted a policy to disclose chemical names and structure formulas as the information on impurities including, in principle, optical enantiomers.

As mentioned in the ICH Q3A guideline, there are cases where the structure determination of impurities is incomplete. Therefore, the chemical structures disclosed in the section Others of monographs include structures that have been determined by NMR, etc., as well as chemically reasonable structures that are estimated from synthetic pathways, etc. At that time, if the stereochemistry is not confirmed, the structure of the relevant part is described using wavy lines, and the hydrogen bonded to the relevant carbon is not described (except when it is essential to show the structure). Furthermore, the chemical name does not include the distinction between *R*-form and *S*-form, and *E*-form and *Z*-form.

The JP-specific consideration may be given to purity tests for organic impurities in drug products in the process listing in the JP. Also in the JP, impurities derived from the products of the reaction between the drug substance and additive/primary packaging are specified as impurities in the drug product. Those impurities are formulation-dependent and may not be formed in different formulations. Since the JP is an official compendium that allows a wide variety of formulations, when it is not appropriate to specify impurities uniformly in the individual monograph, they are subject to the specifications at the time of approval, along with the statement ''Being specified separately when the drug is granted approval based on the Law.''

When the specifications for impurities are reviewed for a new entry of a pharmaceutical product in an individual monograph of the JP, acceptance criteria for impurities may be included in the review according to the following concepts. ICH Q6A guideline point out: Data available upon the marketing application are limited and it has to be taken into consideration that the limited data may influence the design of acceptance criteria. Regarding impurities, since impurity profiles gained during the manufacturing stages may sometimes be different from that gained from development stage, it is stated that changes in impurity profiles at the manufacturing stage should be considered as appropriate. According to this concept, for impurities which should be specified in the process listing in the JP, not only information from development stage but also information about impurity profiles if there are changes at the manufacturing stage, and information at the stage after the product manufacturing becomes stable (hereinafter referred to as the ''stable production stage'') should be taken into consideration.

However, it is undesirable to remove impurities that are present at substantially lower levels, or become undetectable at the stable production stage indiscriminately from the list of candidate compounds to be specified. JP-listed drugs are accepted as drugs by conformance to the specifications in the individual monograph. However, generic drugs, whose manufacturing methods are not necessarily the same as that of the drug substance used for JP monograph, may have different impurity profiles and contain such impurities. Providing information in the process listing in the JP based on the detection results during development stage may result in encompassing impurities found in drug substances and drug products distributed as JP drugs.

Therefore, before the removal of impurities that are present at substantially lower levels or become undetectable at the stable production stage from the JP specification list, the need to establish specifications should be fully examined based on ICH Q3A and Q3B guidelines with respect to safety.

For a drug substance that was approved by the method to identify its impurities with impurity reference materials, it is desirable also in the individual JP monograph, in principle, to establish specifications and test methods appropriately so that the specified impurity becomes identifiable. In regard to impurity control during the manufacturing process, impurities can be controlled by establishing an appropriate control strategy including release testing, in-process tests and process parameters control.

4. References

1) ICH: Guideline for Q3A, Impurities in New Drug Substances.
2) ICH: Guideline for Q3B, Impurities in New Drug Products.

G1 Physics and Chemistry

Change the following as follows:

System Suitability <*G1-2-181*>

In order to ensure the reliability on the results of drug analyses, it is essential to verify that the test method to be applied to the test, including the method prescribed in the Japanese Pharmacopoeia (JP), can give the results adequate for its intended use using the analytical system in the laboratory in which the test is to be performed, then to carry out system suitability testing for confirming that the analytical system maintains the state suitable for the quality test.

1. Definition and role of system suitability

''System Suitability'' is the concept for ensuring that the performance of the analytical system is as suitable for the analysis of the drug as was at the time when the verification of the test method was performed using the system. Usually, system suitability testing should be carried out at every series of drug analysis. The test procedures and acceptance criteria of system suitability testing must be prescribed in the test methods of drugs. The results of drug analyses are not acceptable unless the requirements of system suitability have been met.

System suitability testing is an integral part of test methods using analytical instruments, and based on the concept that the equipments, electronic data processing systems, analytical operations, samples to be analyzed and operators constitute an integral system that can be evaluated, when the test procedures and acceptance criteria of system suitability testing are prescribed in the test methods.

2. Points to consider in setting system suitability

Parameters of system suitability testing to be prescribed in the test method depend on the intended use and type of analytical method. Since system suitability testing is to be carried out in a routine manner, it is preferable to select the parameters necessary for ensuring that the analytical system maintains the state suitable for the analysis of the drug and to prescribe its test procedure able to carry out easily and rapidly.

For example, in the case of quantitative purity tests using liquid chromatography or gas chromatography, the evaluation of parameters such as ''System performance'' (to confirm the ability to analyze target substance specifically), ''System repeatability'' (to confirm that the degree of variation in the analytical results of target substance in replicate injections is within the allowable limit) and ''Test for required detectability'' (to confirm the linearity of chromatographic response around the specification limit) are usually required. However, in the area percentage method, if the influence of a matrix is evaluated and appropriate confirmation of detection is established, such as using a solution with the lowest concentration level that should be controlled in consideration of the property of an analyte, the specification of system repeatability may not be necessary.

The specifications of system suitability in chromatography should be in accordance with Chromatography <*2.00*> or Liquid Chromatography <*2.01*>. The followings are supplements to the section of system suitability prescribed in ''Liquid Chromatography <*2.01*>''.

2.1. System repeatability of HPLC and GC

2.1.1. Allowable limit of system repeatability

It is described in the section of system suitability in ''Liquid Chromatography <*2.01*>'' that ''In principle, total number of replicate injections should be 6'', and ''The allowable limit of ''System repeatability'' should be set at an appropriate level based on the data when suitability of the method for the evaluation of quality of the drug was verified, and the precision necessary for the quality test''. Based on the above description, an allowable limit of system repeatability for 6 replicate injections should be set in consideration with the following descriptions. However, in the case that the test method prescribed in the JP monograph is used for the test, the allowable limit of system repeatability prescribed in the monograph should be applied.

(i) Assay for drug substance (for drug substance with the content nearby 100%): An adequate allowable limit should be set at the level that the chromatographic system is able to give the precision suitable for the evaluation of variation in the content of active ingredient within and among the batches of drug substance. For example, the allowable limit of ''not more than 1.0%'' is usually recommended for the drug substances whose width of content specification are not more than 5%, as is in the case of content specification of 98.0 – 102.0% which is often observed in the assay using liquid chromatography.

(ii) Assay for drug products: An adequate allowable limit should be set considering the width of content specification of the drug product and the allowable limit prescribed in the assay of drug substance (when the drug product is analyzed by a method with the same chromatographic conditions as those used for the analysis of drug substance).

(iii) Purity test for related substances: An adequate allowable limit should be set considering the concentration of active ingredients in the solution used for the system suitability testing. In the case that a solution with active ingredient concentration of 0.5 – 1.0% is used for the test of system repeatability, an allowable limit of ''not more than 2.0%'' is usually recommended.

Recommendations for allowable limits described above should not be applicable to gas chromatography.

2.1.2. Method for decreasing the number of replicate injections without losing the quality of system repeatability testing

It is described in the section of system suitability in ''Liquid Chromatography <*2.01*>'' that ''In principle, total number of replicate injections should be 6. However, in the case that a long time is necessary for one analysis, such as the analysis using the gradient method, or the analysis of samples containing late eluting components, it may be acceptable to decrease the number of replicate injections by adopt-

ing new allowable limit of ''System repeatability'' which can guarantee a level of ''System repeatability'' equivalent to that at 6 replicate injections.'' In consideration of the above description, a method for decreasing the number of replicate injections without losing the quality of system repeatability testing is described below. By utilizing this method, if necessary, one can set the test for system repeatability with reduced number of replicate injections and can change the number once being set.

The following table shows the allowable limits to be attained in the test at 3 – 5 replicate injections (n = 3 – 5) to keep the quality test equivalent to that of test at n = 6.

However, it should be kept in mind that decrease in the number of replicate injections results in increase in the weight of each injection and it becomes more important to maintain the equipment in a suitable state.

Table Allowable limits to be attained in the test at 3 – 5 replicate injections (n = 3 – 5) to keep the quality of test equivalent to that of test at n = 6*

		Allowable limit (RSD)					
Allowable limit prescribed in the test of n = 6		1.0%	2.0%	3.0%	4.0%	5.0%	10.0%
Allowable limit to be attained	n = 5	0.88%	1.76%	2.64%	3.52%	4.40%	8.81%
	n = 4	0.72%	1.43%	2.15%	2.86%	3.58%	7.16%
	n = 3	0.47%	0.95%	1.42%	1.89%	2.37%	4.73%

* The probability for inadequate analytical systems to meet the requirements of system suitability testing, is supposed to be 5%.

Delete the following monograph:

Near Infrared Spectrometry *<G1-3-161>*

Add the following:

Instrumental Measurement of Coloration of Liquids *<G1-4-181>*

This test is harmonized with the European Pharmacopoeia and the U.S. Pharmacopeia.

The parts of the text that are not harmonized among the targeted texts for the harmonization are marked with symbols (◆ ◆), and the texts that are uniquely specified by the JP other than the targeted texts for the harmonization are marked with symbols (◇ ◇).

Information on the harmonization with the European Pharmacopoeia and the U.S. Pharmacopeia is available on the website of the Pharmaceuticals and Medical Devices Agency.

1. Principle

The observed color of an object depends primarily on its light absorbing characteristics. However a variety of conditions such as light-source differences, spectral energy of the illuminant, visual sensitivity of the observer, size differences, background differences and directional differences affect the perception of color. Hue, lightness or brightness and saturation are three attributes of the color. Instrumental measurement under defined conditions allows numerical expression of a color. The base of any instrumental measurement of color is that the human eye has been shown to detect color via three types of receptors.

Instrumental methods for measurement of color provide more objective data than the subjective viewing of colors by a small number of individuals. With adequate maintenance and calibration, instrumental methods can provide accurate, precise and consistent measurements of color that do not drift with time. Through extensive color matching experiments with human subjects having normal color vision, distribution coefficients (weighting factors) have been measured for each wavelength within the wavelength range of the visible spectrum, giving the relative amount of stimulation of each receptor type caused by the light of that wavelength. The International Commission on Illumination (CIE) has developed models taking into account the light source and the angle at which the observer is looking at the target (field of view). In a visual test for color of solution, there are requirements that lead to the use of a 2° angle and diffuse daylight. The mean sensitivity of the human eye is represented by the distribution coefficients $\bar{x}_\lambda$, $\bar{y}_\lambda$ and $\bar{z}_\lambda$ (Fig. 1).

For any color, the amount of stimulation of each receptor type is defined by the set of tristimulus values (X, Y and Z).

The relationship between the distribution coefficients and the tristimulus values (X, Y and Z) is given by the following equations, expressed in terms of integrals. ◇According to the definition in Japanese Industrial Standard Z 8120, the short wavelength limit of the visible light wavelength range can generally be considered to be between 360 – 400 nm and the long wavelength limit is between 760 – 830 nm.◇

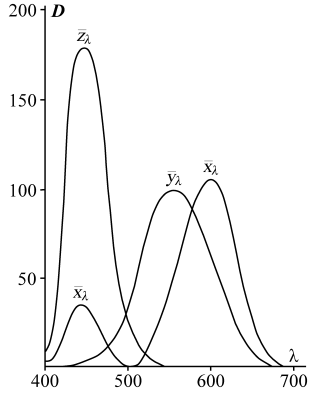


Fig. 1 Mean sensitivity of the human eye represented by distribution coefficients, CIE 2° standard observer (*D*: distribution coefficient; λ: wavelength in nm)

$$X = k\int_0^\infty f_\lambda \bar{x}_\lambda S_\lambda d\lambda$$

$$Y = k\int_0^\infty f_\lambda \bar{y}_\lambda S_\lambda d\lambda$$

$$Z = k\int_0^\infty f_\lambda \bar{z}_\lambda S_\lambda d\lambda$$

$$k = 100/\int_0^\infty \bar{y}_\lambda S_\lambda d\lambda$$

k: normalising constant characterizing the stimulation of one receptor type and the used illumination

S_λ: relative spectral power distribution of the illuminant

$\bar{x}_\lambda$, $\bar{y}_\lambda$ and $\bar{z}_\lambda$: color matching distribution coefficients for CIE 2° Standard Observer

f_λ: spectral transmittance T_λ of the material

λ: wavelength (nm)

In practical calculations of tristimulus values, the integration is approximated by a summation, as follows:

$$X = k\sum_\lambda T_\lambda \bar{x}_\lambda S_\lambda \Delta\lambda$$

$$Y = k\sum_\lambda T_\lambda \bar{y}_\lambda S_\lambda \Delta\lambda$$

$$Z = k\sum_\lambda T_\lambda \bar{z}_\lambda S_\lambda \Delta\lambda$$

$$k = \frac{100}{\sum_\lambda S_\lambda \bar{y}_\lambda \Delta\lambda}$$

The tristimulus values can be used to calculate the CIE *Lab* color space coordinates: L^* (lightness or brightness), a^* (red-green) and b^* (yellow-blue); these are defined by:

$$L^* = 116f(Y/Y_n) - 16$$
$$a^* = 500\,[f(X/X_n) - f(Y/Y_n)]$$
$$b^* = 200\,[f(Y/Y_n) - f(Z/Z_n)]$$

Where

$$f(X/X_n) = (X/X_n)^{1/3} \text{ if } X/X_n > (6/29)^3,$$

otherwise,

$$f(X/X_n) = 841/108\,(X/X_n) + 4/29;$$
$$f(Y/Y_n) = (Y/Y_n)^{1/3} \text{ if } Y/Y_n > (6/29)^3,$$

otherwise,

$$f(Y/Y_n) = 841/108\,(Y/Y_n) + 4/29;$$
$$f(Z/Z_n) = (Z/Z_n)^{1/3} \text{ if } Z/Z_n > (6/29)^3,$$

otherwise,

$$f(Z/Z_n) = 841/108\,(Z/Z_n) + 4/29.$$

X_n, Y_n and Z_n, are the tristimulus values of *purified water*.

In the spectrophotometric method, transmittance values are obtained at discrete wavelengths throughout the visible spectrum. These values are then used to calculate the tristimulus values through the use of weighting factors $\bar{x}_\lambda$, $\bar{y}_\lambda$ and $\bar{z}_\lambda$ for a 2° Standard Observer and CIE standard illuminant C (see the International Commission on Illumination publication, CIE).

2. Spectrophotometric method

Operate a suitable spectrophotometer according to the instructions of the manufacturer and determine the transmittance T at least from 400 nm to 700 nm, at intervals of not greater than 10 nm. Express the result as a percentage (%). Calculate the tristimulus values X, Y, and Z and the color co-ordinates L^*, a^* and b^*.

3. Determination of coloration

Calibrate the instrument as per the instrument manufacturer's recommendation. System performance tests are done prior to each measurement or at regular intervals, depending on the use of the apparatus. To this purpose, use certified reference materials (Certified filters or certified standard solutions recommended by the instrument's manufacturer.) within the measurement range.

Operate the apparatus according to the manufacturer's instructions and measure the test solution and standard solution(s) under the same conditions (e.g. path length of the cuvette, temperature).

For transmittance measurements *purified water* is used as standard and assigned a transmittance of 100.0% at all wavelengths throughout the visible spectrum.

Then the weighing factors $\bar{x}_\lambda$, $\bar{y}_\lambda$ and $\bar{z}_\lambda$ for CIE standard illuminant C are used to calculate the tristimulus values corresponding to color co-ordinates $L^* = 100$, $a^* = 0$ and $b^* = 0$.

Reference measurements can be made using the color co-ordinates of *purified water* or freshly prepared matching fluids for color, or using the respective color co-ordinates stored in the instrument manufacturer's database, provided the latter have been obtained under the same testing conditions.

If the test solution is turbid or hazy, it is filtered or centrifuged. If the test solution is not filtered or centrifuged, any haziness or turbidity is reported with the results. Air

bubbles are to be avoided and removed.

The instrumental method is used to compare two solutions in respect to their color or color difference, or a deviation from a defined color. Calculate the color difference between the test solution t and a matching fluid for color r as ΔE^*_{tr} using the following equation:

$$E^*_{tr} = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2}$$

where ΔL^*, Δa^* and Δb^* are the differences in color coordinates.

Instead of the color coordinates CIE *Lab*, the color coordinates CIE *LCh* may be used.

4. Assessment of location within the $L^*a^*b^*$ color space

Instruments may provide information on the actual location of the test solution within the $L^*a^*b^*$ color space. Using appropriate algorithms, correspondence to matching fluids for color (such as ''test solution equals matching fluid for color XY'', ''test solution close to matching fluid for color XY'' or ''test solution between matching fluids for color XY and XZ'') can be obtained.

Add the following:

Control Strategies and Change Control Concepts at Each Stage of Chromatography Lifecycle (Change Control in Chromatography Lifecycle) *<G1-5-181>*

Analytical methods (analytical procedures) for pharmaceuticals must be set to provide test results suitable for their purpose, which must be considered throughout the lifecycle of analytical procedures, from design to development, qualification and continuous verification. In the field of drug development, particularly in the fields of manufacturing control and quality control, the effort of systematic quality assurance by quality risk assessment is implemented throughout the lifecycle (General Information ''Basic Concept of Quality Risk Management'' *<G0-2-170>*). Effort to apply similar approaches to control strategy at each stage of the life cycle of analytical procedures are described.[1)-4)]

Various chromatographic systems are widely used for the analysis of pharmaceuticals, their components and impurities. Under these circumstances, a guide for changing analytical conditions was presented in the internationally harmonized test methods using chromatography (Chromatography *<2.00>*). However, there are various causes and timings for changing analysis conditions, and the positions of these factors in the overall lifecycle should be considered when change control of analytical condition is designed. Therefore, this general information describes the outline of the methodology for establishing control strategy at each stage of a chromatography lifecycle, aiming at the efficient control of analytical procedures, including changes of analytical methods. The methodology described below does not intend to newly add or mitigate regulatory requirements, but can be apprehended as the systematic documentation of the work that has been performed in laboratories. In addition, the concept of change control described in this General Information can be used as a reference for quality tests of pharmaceuticals in public testing institutions.

1. Analytical procedures that give test results suitable for the purpose of the test

Before designing and developing an analytical procedure, the purpose and goal (target profile) for the development of the analytical procedure are provisionally set and finalized in the latter stage of the development. When chromatography is used for quantitative analysis of active ingredients, etc., an analyte must be quantified with an accuracy and a precision within a certain range including the labeled amount in the presence of impurities or excipients. In addition, quantitative tests for impurities must be able to quantify impurities with an accuracy and a precision in the presence of various components presented in a sample within a range from the reporting threshold[5)] to 120% of the specification limit. As stated in section 5, for example, an analytical procedure may be changed or an analytical procedure itself may become unnecessary due to changes in impurity profiles, etc., however, the target profile of this analytical procedure can be the indicator whether the analytical performance characteristics are appropriate over the lifecycle. Here, the analytical performance characteristics are mainly characteristics evaluated by the ''validation characteristics'' described in General Information ''Validation of Analytical Procedures'' *<G1-1-130>*. (In the test methods prescribed in the Japanese Pharmacopoeia, specifications and acceptance criteria in the monographs can be a target profile.)

2. Design and development of the draft procedure of chromatography

When the target profile of an analytical procedure is proposed, the draft of the analytical procedure is designed based on this profile, and the analytical procedure is established. In the process of the establishment, the implementation of risk assessment deepens the understanding of sources of variability in a series of analytical operations including analytical systems and their effect on reported values. Sources of variability are investigated using a method such as a characteristic diagram (Ishikawa diagram), and the root causes are identified and eliminated. At that time, the justification of various relevant validation characteristics proposed in the target profile, such as accuracy and precision, as well as specificity and linearity that affect the accuracy and precision, is confirmed. By a series of the confirmation of the justification, the target profile of the analytical procedure is reflected in key analytical performance characteristics[1)], and at the same time, it is possible to identify sources of variability and modify the analytical method from the results of those experiments. In addition, design of experiments (DOE), etc. can be used to clarify the relationship among the sources of variability and to study

the degree of the variation that can occur when the analytical procedure is conducted under different conditions. Then, the sources of variability to be controlled and the acceptable ranges are clarified, and the analytical procedure is optimized. Appropriate experimental results obtained during the establishment of the analytical procedure could be used as a substitute for validation data.

Establish a control strategy based on the results of risk assessment. Control items may also include, for example, temperature, stability of sample solution, and number of replicates as well as the requirements of system suitability as described below.

System suitability testing is set as an appropriate check test to evaluate the effect of the sources of variability remaining in the analytical procedure that cannot be controlled as variable sources of variability(e.g., pH of mobile phase and column size) (General Information ''System Suitability'' *<G1-2-181>*). Therefore, system suitability testing should be considered as a minimum control method during the qualification stage of analytical performance described below. System suitability testing should be set to focus on the analytical performance characteristics that can be affected and to ensure that the testing is considered to meet the requirements of the target profile. For system suitability testing, for example, resolution and a symmetry factor are set.

3. Preparatory stage for qualification

A control strategy for an analytical method is proposed by the clarification of the sources of variability and accumulated knowledge, and the analytical performance is ready to be qualified.

When a test method is already prescribed in the Japanese Pharmacopoeia, based on the test method, it is necessary to understand and examine beforehand to what extent additional sources of variability exist in the laboratory where the actual analysis is conducted and to what extent advance information has been already obtained. Additional sources of variability include, for example, samples, reagents, facilities, instruments, and the number of replicates that can occur with those variations. When applying a test method prescribed in the Japanese Pharmacopoeia, in many cases analysts do not have the knowledge and understanding obtained during the development of the analytical method. Therefore, the analysts should be aware of the potential risks due to additional sources of variability and should ensure that the above risks are appropriately reduced by the qualification of the analytical performance, etc. (Column information available on the Pharmaceuticals and Medical Devices Agency website may be useful as advance information.)

4. Qualification of analytical procedure performance

The purpose of qualification is to confirm that an analytical procedure constantly meets a target profile in a routinely used laboratory. For qualification testing, a protocol is prepared and the test is performed according to the procedure manual and appropriate control. As the result of the test, for example, when the variation of the reported values may exceed the requirements in the target profile, examine whether the control strategy is optimized for the laboratory, identify the sources of variability, and the control strategy of the analytical method may be improved or revised. If an analytical method was developed in a routinely used laboratory, the qualification of analytical performance could be omitted.

Even when applying a test method prescribed in the Japanese Pharmacopoeia, different control strategies are required for different laboratories and instruments. For qualification in the laboratory where a test method prescribed in the Japanese Pharmacopoeia is performed, the process of the quality risk management of the analytical method should be considered to meet the intended target profiles of specifications and acceptance criteria in each monograph.

In the qualification when applying test methods prescribed in the Japanese Pharmacopoeia, it is not essential to perform the verification of the validity of validation characteristics again to the same extent when establishing the analytical procedures, however, it is necessary to confirm the qualification using appropriate validation characteristics listed in General Information ''Validation of Analytical Procedures'' *<G1-1-130>*. The content of the implementation should consider the type of analytical procedures, related instruments, etc. In addition, consideration should be given to factors derived from test samples. For example, when applying a test method prescribed in the Japanese Pharmacopoeia, impurities that may differ depending on a drug substance or drug product can affect the ''specificity'' of the test method. When resolution is set in the system suitability testing, confirm the effect by the resolution, and if the specificity is reduced, examine the effect on the test result. If the analytical performance deteriorates, it will be necessary to examine the analytical conditions. In addition, since different excipients in drug products may affect interference with a substance to be analyzed (specificity), detection (detection limit), recovery (accuracy) and variation in quantitative values (precision), perform the qualification using system suitability testing and appropriate validation characteristics described in General Information ''Validation of Analytical Procedures'' *<G1-1-130>*.

5. Continuous verification of Analytical Methods

1) Routine monitoring: At this stage, data on the performance of analytical procedures, such as analytical results, suitability for system suitability, deviations from specifications and specific trends, are collected and analyzed. If nonconformity to the system suitability, deviation from the specification, or a specific trend becomes clear, it is necessary to examine the cause and take corrective and preventive measures.
2) Change of analytical procedures: As with the manufacture of pharmaceuticals, analytical procedures may be changed for the activity of continual improvement and for analysis in different environments. When newly applying a test method prescribed in the Japanese Pharmacopoeia, it may be necessary to change the procedure according to

the current equipment or columns. Furthermore, it is expected that an analytical method will need to be changed as the result of routine monitoring described in 1). Depending on the extent of the change, the contents and amount of work for evaluating the effect of the change on the test results vary. Examples of possible changes are shown below.

① When an analytical procedure is changed within the acceptable range of the procedure evaluated at the time of the development of the analytical procedure, it is necessary to evaluate the effect on a case-by-case basis and confirm that the changed procedure always meets the target profile. (However, this does not apply when such acceptable range has not been examined at the time of the development of the analytical method.) Even if the change of each condition is within the acceptable range, when multiple conditions are changed, it may be necessary to take similar measures as the following ②.

② When an analytical procedure is changed beyond the acceptable range of variability of the procedure evaluated at the time of the development of the analytical method, risk assessment is required. In addition, if the acceptable range of changes has not been examined by quality risk management at the time of the development of the analytical method, risk assessment is required when changing the analytical conditions. When conducting risk assessment, consider which analytical performance characteristics (validation characteristics) can be affected by the change. Then, qualification is performed to confirm that the analytical performance does not deviate from the target profile (refer to 4). Specifically, verify using validation characteristics that may be affected by the change among validation characteristics listed in General Information ''Validation of Analytical Procedures'' *〈G1-1-130〉*. When validation characteristics that may be affected by a change are set as one item of system suitability testing, the validation characteristics may be verified by using the system suitability testing. Further, when changing a column size and the composition of a mobile phase in chromatography, verify analytical performance appropriately, referring to ''Adjustment of Chromatographic Conditions'' in Chromatography *〈2.00〉*.

③ When a laboratory is changed or a test method prescribed in the Japanese Pharmacopoeia is newly applied, the analytical performance characteristics may be affected by the change in analytical instruments, analysts, reagents, etc., so perform risk assessment and appropriate qualification (refer to 3 and 4). On the other hand, when updating analytical equipment or columns or replacing analysts in a same laboratory, at least perform system suitability testing with the changed analytical system to confirm that the same results are obtained before and after the change.

④ When changing to a new analytical procedure or technology, qualification must be performed during the development of the new analytical procedure (refer to 2, 3 and 4) to demonstrate that the new procedure meets the target profile.

⑤ When a change that affects a target profile (e.g., changes in specifications, changes to methods for determining the amount of a new analyte, such as impurities that were not considered in the original target profile) is required, it may be necessary to review the current analytical procedure and qualification to update the target profile and assess whether the analytical procedure meets the requirements of the new target profile (refer to 1, 2, 3 and 4).

The extent of work to confirm whether a change in an analytical method gives a test result suitable for the purpose depends on ① risk associated with the change, ② knowledge obtained about the analytical procedure, and ③ control strategies. Whatever changes are made, perform more or less risk assessment to ensure that the changed analytical procedure provides the results that meet the purpose of the test method (i.e., within the range specified in the target profile).

6. References

1) G.P. Martin, et al., Pharmacopeial Forum 39 (5), 2013
2) Proposed New USP General Chapter: The Analytical Procedure Lifecycle 〈1220〉, Pharmacopeial Forum 43 (1), 2017
3) K.L. Barnett, et al., Pharmacopeial Forum 42 (5), 2016
4) E. Kovacs, et al., Pharmacopeial Forum 42 (5), 2016
5) ICH: Guideline for Q3A (R2), Impurities in New Drug Substances.

G2 Solid-state Properties

Add the following:

Measurement of Powder Flow Properties by Shear Cell Methods *〈G2-5-181〉*

In the manufacturing of pharmaceuticals, a large number of processes involve powder transfer and feeding such as putting raw materials into a mixer and filling a powder into the mortar of a tableting machine. Since powder flowability is related to preparation characteristics such as mass and content uniformity, it greatly affects the product quality. It is important to evaluate powder flow properties in the design of formulations, processes and pharmaceutical production equipment. Since shear cell methods are among the most important methods for measuring powder flow properties and can be performed under a wide variety of stress conditions, parameters useful for predicting various powder behaviors during the manufacturing of pharmaceuticals, such as an angle of critical state line, unconfined yield strength and flow factor, can be determined.

1. Principle

A powder in a hopper, etc. may not immediately flow due to adhesion/coagulation of particles and interference to the mutual motion by complex surface shape when shear stresses are applied from the outside. When a sufficiently large shear stress is attained, the powder suddenly starts to flow. In addition, powder flow under quasi-static conditions such as flow in a bin strongly depends on the consolidation stresses. Consolidation is an operation to apply a load to a powder bed to reduce the bulk volume to change the bulk density or the void fraction of the powder bed. The shear cell methods are the tests to determine the flow properties of a powder in the process of transition from a static state to a non-static state when the powder is sheared by applying a normal stress. In other words, a maximum shear stress immediately before fail and a dynamic friction force in a steady-state flow are measured.

Powder flowability under compressed conditions is governed by three factors: the degree of consolidation (bulk density or void fraction, ε), normal stress (σ) and shear stress (τ). A three dimensional representation of the applied normal stress, shear stress and void fraction is called the Roscoe condition diagram (Fig. 1), and the shear cell methods are test methods to obtain the Roscoe condition diagram or yield loci which constitute the Roscoe condition diagram.

2. Apparatus

The shear cell methods can be performed under constant load or constant volume conditions. In both conditions, apparatuses typically consist of a shear cell, weights or a press machine for applying normal stress to a sample, a mechanism for shearing a sample, and load cells for measuring normal stresses and shear stresses.

2.1. Shear cell

Many shear cells have the structure which can make a shear plane somewhere in a powder bed by shearing a powder filled in a container dividable into two parts, upper and lower cells, while applying a normal stress. In the constant load conditions, a lid that fits within an upper cell is free to move up and down when a shear stress is applied, and the volume of the powder container changes. In the constant volume conditions, the position of the lid is fixed by pressing the lid with a press machine, etc.

The shear cells are classified into two types according to the motion that provides a shear stress; translational or rotational.

2.1.1. Translational shear cell

In the translational shear cell, one of the upper and lower cells is fixed and the other is moved horizontally (translationally) to apply a shear stress to the powder bed filled in the two cells. The shear plane forms at the boundary between the powder contained in the lower cell and the powder contained in the upper ring cell. Some translational shear cells are cylindrical (Fig. 2), and others are sandwiching a sample between two plates on top and bottom and with no side walls. The representative example of the former is the Jenike shear cell, and that of the latter is a parallel-plate cell.

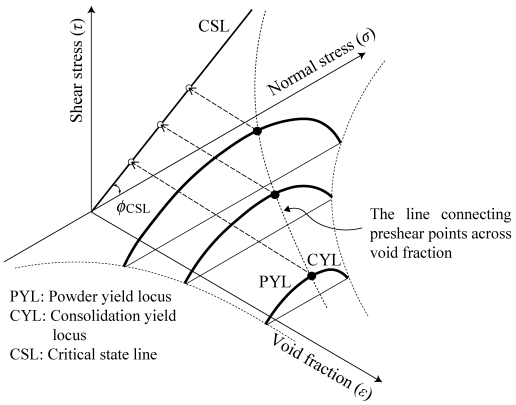


Fig. 1 Roscoe condition diagram

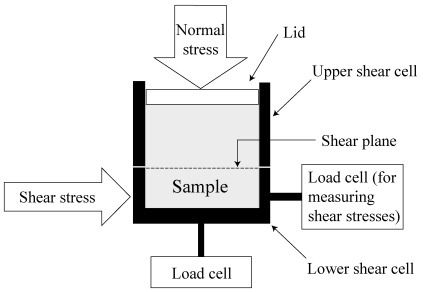


Fig. 2 Example of translational shear cell

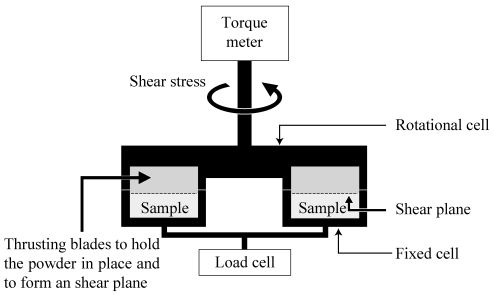


Fig. 3 Example of rotational shear cell

2.1.2. Rotational shear cell

In the rotational shear cell, one of the upper and lower cells is fixed, and the other is rotated to apply a shear stress to the powder bed filled in the two cells. Some rotational shear cells are cylindrical and others are annular (Fig. 3). Any rotational shear cells usually have surface features that prevent the powder from sliding at the cell surface. Several blades are radially attached on the side of the shear cell where it contacts the sample, so that the powder is hold in place by the thrusting blades. A shear plane forms in the powder bed directly under the blades when the shear cell is rotated.

2.2. Other components

A load cell is a device using a spring, a piezoelectric element, etc. to detect a load or torque and converts the applied force into an electrical signal. The load cells and weights for applying a normal stress to a sample must be regularly calibrated with standards with measurement

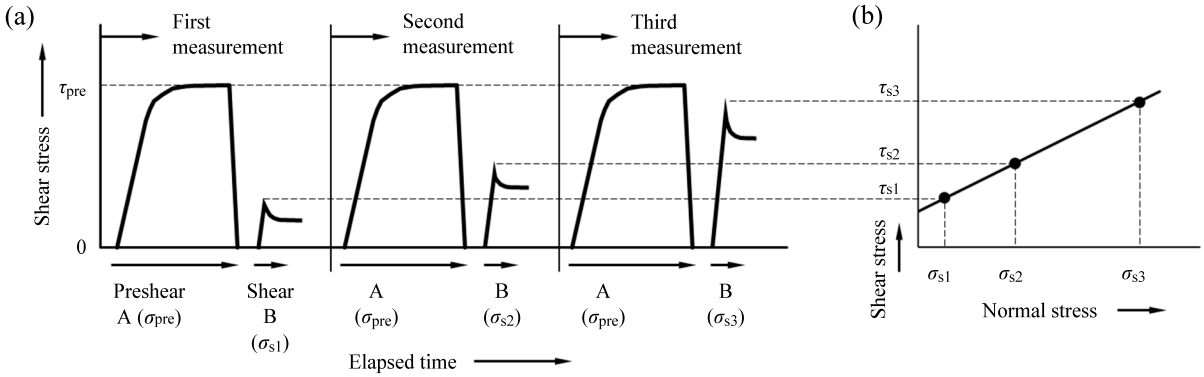


Fig. 4 Plots of shear stress versus time during the test procedure (a) and corresponding powder yield locus (b)

traceability.

3. Measurement

A temperature of 20 ± 5°C and a relative humidity of 50 ± 10% are recommended as measurement environment. A fresh powder samples should be used for each test. However, this does not apply to samples clearly having no consolidation history or rare samples, if the description of their reuse is recorded. Gently fill the shear cell with a sample of powder using a spatula or a sieve with an opening size larger than the maximum particle size of the sample. At this time, care should be taken not to form cavities in the powder bed. The surface of the filled sample is leveled with a spatula, etc. Under the constant load conditions, first the powder sample is consolidated (preshear) in order to perform a test with a desired constant void fraction during one measurement.

The test procedure under constant load conditions using the Jenike shear cell, etc. is shown in Fig. 4 by a pattern diagram. Prior to a test, the powder sample is sheared with applying a preconsolidation stress (σ_{pre}) until a shear stress reaches a steady value (τ_{pre}) for preshear (Fig. 4 (a) A). Under the constant load conditions, the volume of powder can decrease or increase during the preshear and becomes constant when a steady-state is achieved. In other words, the void fraction of the powder bed where the shear stress became constant under certain normal stress conditions is uniquely determined by the powder flow properties. In the following main test, measurements are performed on the sample having this void fraction. After the shear stress is reduced to zero, the normal stress acting on the sample is decreased from σ_{pre} to a new value (σ_{sx}, $x = 1, 2, 3\cdots$) for the next step of the test procedure (Fig. 4 (a) B). When the shear stress is gradually increased, the maximum shear stress measured immediately before the consolidated powder starts to flow is τ_{sx} ($x = 1, 2, 3\cdots$). The A-B procedure is repeated at 3 to 5 points of σ_{sx} which is less than the normal stress at preshear (σ_{pre}), and the powder yield locus (PYL, Fig. 4 (b)) is obtained by plotting the results.

On the other hand, under the constant volume conditions, shear stresses are continuously measured with progressively changing a normal stress while the void fraction is kept at a specified value by a press machine, etc. controlling the lid position. Since the void fraction is constant under the constant volume conditions, the consolidation yield locus (CYL) in Fig. 1 is obtained during the process of consolidation failure. The PYL and CYL share a preshear point and are combined to form a single yield locus (YL).

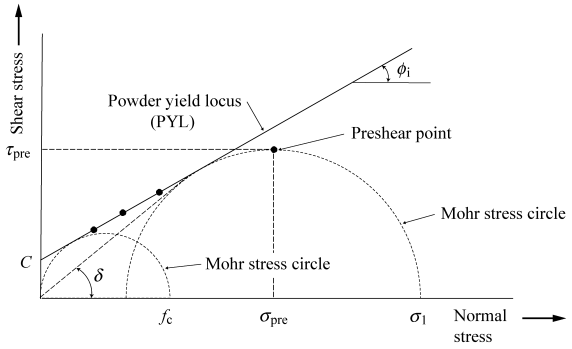


Fig. 5 Graphical representation of various parameters obtainable from PYL

4. Data analysis

Shear stresses can be measured in both states where a powder is not flowing (static state) and where a powder is flowing (dynamic state).

The approximate line connecting the points (σ_{sx}, τ_{sx}) shown in Fig. 4 (b) represents the relationship between the shear stress and normal stress immediately before the consolidated powder sample starts to fail, namely, the relationship in a static state, and is referred to as a PYL. Further, the point (σ_{pre}, τ_{pre}), where the shear stress τ_{pre} reached constant by applying the preshear normal stress σ_{pre}, is added to Fig. 4 (b) (Fig. 5). This point is measured in a dynamic state and is called a preshear point. Next, two circles having a center on the normal stress axis are drawn: The first is a circle passing through the preshear point and tangential to the PYL (the larger semicircle in Fig. 5). The second is a circle passing through the origin and tangential to the PYL (the smaller semicircle in Fig. 5). The circles having a center on the normal stress axis and tangential to a PYL are called the Mohr stress circles.

Various parameters that describe powder flowability can be obtained from a PYL and Mohr stress circles.

4.1. Shearing cohesion (*C*)

The failure shear stress at zero normal stress, normally obtained by extrapolation of the PYL. An indication of the intrinsic strength of an unconfined powder.

4.2. Angle of internal friction (φ_i)

The angle formed by the PYL and the σ axis. The inclination (tan φ_i) of the PYL indicates the internal friction between the powder particles under the consolidation conditions measured.

4.3. Effective angle of internal friction (δ)

The angle formed by the straight line which passes through the origin and tangential to the larger Mohr stress circle in Fig. 5, and the σ axis. The angle may be used as a relative indication of the internal friction when the powder flow is in a steady-state.

4.4. Flow function (FF)

The ratio (σ_1/f_c: ff_c) of the maximum principal stress (σ_1) of the larger Mohr stress circle and the maximum principal stress (uniaxial collapse stress: f_c) of the smaller Mohr stress circle in Fig. 5 may be used as a quantitative classification indication of powder flowability (Table 1). A regression line obtained from the σ_1-f_c relationship measured under various consolidation conditions for one material, which is called FF, is used for powder flow analysis such as when designing a hopper.

Table 1 General classification of flowability

ff_c	Flowability
< 1	Not flowing
1 – 2	Very cohesive, difficult-flowing
2 – 4	Cohesive, slightly difficult-flowing
4 – 10	Easy-flowing
10 <	Free-flowing

It should be noted that even a same powder shows different flowability if the degree of consolidation is different because the above parameters can be determined from Fig. 5 where the measurements were performed with a sample having one specified void fraction.

On the other hand, the critical state line (CSL) in Fig. 1 is obtained by projecting the preshear points (black circles in the figure), which are determined from several samples having a different void fraction, onto the σ-τ plane, and is a straight line passing through the origin. Since the CSL shows the normal stress-shear stress relationship in a dynamic state, it reflects the powder flow properties without depending on the type of apparatus used for measurement. The angle formed by the CSL and the σ axis is called the angle of critical state line (φ_{CSL}); the smaller the value, the higher the flowability.

5. Report of results

Measurements under the same conditions are repeated an appropriate number of times according to the variation in the obtained values, and the average value is reported along with the items listed in Table 2.

Table 2 Examples of items to be described in the report of results

Item	Content
General information	Measurement date/time, name of operator, sample name, apparatus used (type, model/manufacturing company) and type of cells, measurement method (constant load method or constant volume method), etc.
Sample-related information	Particle size and particle size distribution, method of particle size measurement, bulk density, water content, conditions for drying, etc.
Measurement conditions	Temperature and relative humidity during measurements, size of cells used, sample amount, preconsolidation conditions, shear rate, etc.
Results	Normal stress and shear stress of each measurement in the main test, σ-τ plot showing yield locus, various parameters obtained by the analysis of angle of critical state line, etc.
Other special notes	Descriptions when the measurement conditions such as the preconsolidation stress and the number of measurements are changed from the normal setting, or when the sample is reused, etc.

G4 Microorganisms

Add the following:

Biorisk Management of the Handling of Microorganisms in Microbial Tests <*G4-11-181*>

This general information describes basic requirements in the safe handling of microorganisms to be considered in performing the microbial tests in the General Tests (4.02 Microbial Assay for Antibiotics, 4.05 Microbiological Examination of Non-sterile Products, 4.06 Sterility Test), the Test for Crude Drugs (5.02 Microbial Limit Test for Crude Drugs and Preparations containing Crude Drugs and Preparations containing Crude Drugs as Main Ingredient), the tests in the General Information G3 Biotechnological/Biological products (Basic Requirements for Viral Safety of Biotechnological/Biological Products listed in Japanese Pharmacopoeia <*G3-13-141*> and Mycoplasma Testing for Cell Substrates used for the Production of Biotechnological/Biological Products <*G3-14-170*>) and the tests in General Information G4 Microorganisms (Preser-

vatives-Effectiveness Tests <*G4-3-170*>, Rapid Microbial Methods <*G4-6-170*>, Rapid Identification of Microorganisms Based on Molecular Biological Method <*G4-7-160*>, Rapid Counting of Microbes using Fluorescent Staining <*G4-8-152*> and Disinfection and Decontamination Methods <*G4-9-170*>).

In the work of handling microorganisms, it is required to manage accurately biorisk generated by performing tests. Since risks while handling microorganisms vary depending on the characteristics of microorganisms and the contents of work handled, it is necessary to perform risk assessment for each work to identify, analyze and evaluate the risks, to protect workers handling microorganisms and also to reduce risks in laboratory biosafety and biosecurity. In the practice, a responsible person and a person in charge related to biorisk management should be assigned in the organization, and rules and plans for the operation should be established by them. In order to reduce risk, measures for laboratory biosafety should be performed by combining four elements: safety control, personal protective equipment, safety equipment, and physical containment facilities/equipment. The established risk management method should be updated through continuous risk reviews[1)].

The basic concept necessary for biorisk management in the handling of microorganisms is shown below.

1. Explanation of terms

The definitions of terms used in this general information are as follows.

1.1. Laboratory: Facilities/equipment that handle microorganisms for the purpose of performing experiments for inspection, testing, studies, etc.

1.2. Biohazard: A disaster caused by organisms and biological products.

1.3. Classification of microbiological risk level: A classification of the risk of microorganisms to workers handling microorganisms and related persons.

1.4. Laboratory Biosafety: Risk mitigation measures according to biohazard risks are called biosafety. The purpose is to prevent the unintentional exposure, spread and accidental leaks of pathogens or toxins. Among them, laboratory biosafety should be performed by combining four elements, safety control, personal protective equipment, safety equipment and physical containment facilities/equipment.

1.5. Laboratory Biosafety Level (BSL): BSLs are classified from BSL1 to BSL4 by combining the four elements that practice laboratory biosafety, and risk mitigation measures according to each BSL should be constructed.

1.6. Biosecurity: Biosecurity means prevention and control in laboratories in order to protect unauthorized access, loss, theft, overuse, misuse, diversion and intentional release of Valuable Biological Materials which need protection/monitoring.

1.7. Biorisk: Biorisk is the merged risk of laboratory biosafety and biosecurity, and includes all probability and chance where harmful events (accidental infection, unauthorized access, loss, theft, overuse, misuse, diversion or intentional release) will occur.

1.8. Biorisk Management: Biorisk management consists of three elements: risk assessment, risk mitigation and performance.

1.9. Worker handling microorganisms: A person who handles microorganisms directly in a laboratory and a person who enters a laboratory to maintain laboratory facilities.

1.10. Related person: A person who has a possibility of infection, such as a laboratory user who contact with a worker handling microorganisms, a colleague or a housemate of a worker handling microorganisms.

1.11. Good Microbiological Technique (GMT): Standard techniques for the safe handling of microorganisms. It includes the preparation of educational programs for acquiring technology, standard work procedures and rules.

1.12. Personal Protective Equipment (PPE): A set of tools worn by individuals to protect workers handling microorganisms against exposure of biohazardous substances. For example, masks, respiratory protection tools, goggles, gloves, protective clothing, shoe covers, etc.

1.13. Safety Equipment: A set of apparatus, instruments, and devices that protects workers handling microorganisms from exposure to biohazardous substances. For example, electric pipettes, sealed containers, biological safety cabinets, etc. A biological safety cabinet is to protect workers, laboratory environment and work materials from exposure to infectious aerosols. There are mainly two types, an opened type which isolates the inside and outside by air barrier and a sealed glove box type.

1.14. Physical containment facilities/equipment: Physical containment facilities/equipment are classified into 4, physical containment level P1, P2, P3, and P4. The object of the facilities/equipment is to provide workers with safe handling of hazardous substance according to the classification of the risk level.

1.15. Controlled area: An area where biorisk management is required. The area includes not only laboratories for handling microorganisms, but also waste treatment facilities/equipment, wastewater treatment facilities/equipment, air conditioning machine rooms, etc. that have biohazard risk.

2. Risk assessment in handling microorganisms

The following risks accompanying the handling of microorganisms in each execution plan of tests should be evaluated.

2.1. Risks concerning with laboratory biosafety

2.1.1. Risks due to the characteristics of microorganisms

(i) Risks based on the classification of microbiological risk level

Microorganisms have a different extent of harm to humans depending on their species or strain. Considering the symptoms of workers handling microorganisms when infected with microorganisms and impact on related persons, microorganisms are classified to the microbiological risk levels 1 to 4 (Table 1) in descending order of the risk. The classification of individual microbiological risk level differs according to country/region, target (human or livestock), presence of therapeutics or prophylaxis, minimum infective

dose, route of infection, amount used, work contents, etc. Microorganisms that do not exist in Japan are often classified as high risk levels, when it is handled in Japan.

(ii) Risks due to the routes of microbial infection and exposure

Consider both the infectious route of microorganisms used and the supposed exposure to workers according to handling methods. In natural infection, oral cavity, nasal cavity and ocular mucosa are likely to be routes of infection, and contact with mucosa, oral infection, droplet infection, aerial infection, presence of insect vectors, etc. should be considered. In laboratory infection, attention should be paid to needle-stick infection, infection from skin wounds, and infection caused by contact with contaminants such as laboratory tools.

(iii) Risk due to host sensitivity

The different risks of susceptibility of workers handling microorganisms to microorganism should be considered. As a preventive measure for infection, vaccination can provide resistance to workers handling microorganisms and reduce the risk of infection.

(iv) Risks by using microorganisms specified in relevant laws and regulations

When using, possessing, storing and transporting microbial species, strains and toxins specified by the laws[2-5], comply with the relevant laws. For general matters, refer to the laws, notices and administrative communications that describe them in detail.

2.1.2. Risks from handling operations

(i) Risks due to the form and amount of microorganisms handled

Pipetting and other laboratory operations often generate droplets and aerosol, and aerosol containing microorganisms have a high risk of being diffused extensively by air currents. Take into account that as the amount of microorganisms and toxins handled increases, the associated risks increase.

(ii) Risks due to the skill of workers handling microorganisms

Consider that work by persons who do not have sufficient knowledge about microorganisms to be handled or who have not received sufficient education and training on how to handle microorganisms appropriately becomes to be high risk.

(iii) Risk due to the shape of tools handled

Considering that using glassware for work increases not only the risk of contamination due to breakage but also the risk of infection through wounds caused by breakages, examine the purpose when using glassware.

(iv) Risks accompanying work contents

Consider that opening containers containing liquid or a powder, handling liquid using a pipette or pipetter, stirring liquid with a vortex mixer or transporting a supernatant after centrifugation to another container may increase the risk of generating aerosol.

(v) Risks in each work process

If there are multiple work processes, consider that the risk varies depending on the work contents of each process.

(vi) Risks on accepting/dispensing microorganisms

Consider new risks arising from the acceptance and dispensation of microorganisms, strains and toxins.

(vii) Risks on transporting microorganisms

When transporting samples containing microorganisms, consider that the risk (impact on external) differs between transporting within controlled areas and transporting to the outside of controlled areas.

(viii) Risk of infectious wastes

All tools and samples contaminated with microorganisms during work should be handled as infectious waste having the risk of infection until they are decontaminated (disinfection or sterilization) to inactivate microorganisms.

(ix) Risks in case of emergency

Consider emergency response when workers handling microorganisms are exposed to microorganisms, contamination of facilities/equipment, leakage of microorganisms to the outside of facilities, etc.

2.2. Risks concerning with biosecurity

When entrance management to microorganism handling facilities and a storage management method of microorganisms are not properly carried out, illegal access to microorganisms, loss, theft, overuse, misuse, diversion, intentional release, etc. become risks in biosecurity.

3. Risk mitigation measures in handling microorganisms

For each risk clarified by evaluation, necessary measures should be taken to reduce the risk to workers handling microorganisms and related persons. The following contents are necessary.

3.1. Establishment of biorisk management system

Institutions that possess and handle microorganisms are required to establish a management organization for biorisk management regardless of the number of workers handling microorganisms[6-8].

- Clarify roles, authorities, and responsibilities in the management organization.
- Assign a responsible person for biorisk management.
- Assign a person in charge of biorisk management.
- Establish rules and plans for biorisk management.

The contents to be performed include the following.

- Reduce risks in laboratory biosafety.
- Reduce risks in biosecurity.
- Carry out education and training for biorisk.
- Establish and carry out a maintenance plan for facilities/equipment in controlled areas.
- Comply with relevant laws and regulations.

3.2. Mitigation of risks concerning with laboratory biosafety

There are four main elements in risk mitigation measures concerning handling microorganisms: safety control, personal protective equipment, safety instruments/devices and physical containment facilities/equipment. Reduce risks by performing laboratory biosafety measures (Table 2) that combine the four elements according to biorisk[9].

(i) Safety Management

Safety management includes all related matters and requires the following:

- Establish rules for various items necessary for the safe

handling of microorganisms.

- Prepare standard operation procedures based on good microbiological technique (GMT).
- Continue education and training in order to learn good microbiological technique (GMT).
- If there are effective preventive measures such as vaccines against microorganisms to be used for the health management of workers handling microorganisms, introduce a system to utilize the vaccination history of workers handling microorganisms.
- Establish emergency measures.
- Carry out education and training for biorisk.

(ii) Personal protective equipment

Use appropriate personal protective equipment (PPE) during work to reduce the risk of microbial exposure. Select the appropriate personal protective equipment (PPE) according to the characteristics and infectious routes of microorganisms to be handled, and work contents.

(iii) Safety equipment

Use an electric pipette, etc., so that workers handling microorganisms do not contact with microorganisms directly. Use non-leaking tools/devices made of materials that are not easily damaged. When disposing sharp tools such as injection needles, dispose them in containers (such as needle collection containers) that do not allow them to penetrate.

Microorganisms should be handled in biological safety cabinets, etc. to reduce the risks of exposure to microorganisms contained in aerosol generated and their spread to workplaces. For samples with high risk of aerosol infection, use a centrifuge applied with measures to confine aerosol. Safety equipments used in a biological safety cabinet should be taken out after decontamination in the biological safety cabinet.

Microorganisms (including bacterial spores and fungal spores) should not be handled in a clean-bench etc. where containment capability is not guaranteed.

(iv) Physical containment facilities/equipment

Define a risk level by risk assessment based on the characteristics of microorganisms and work contents, and use necessary physical containment facilities/equipment. For facilities/equipment there are requirements specified for each containment level[10,11], and facilities/equipment being physical containment level 3 (P3) or higher are required to take effective measures to prevent workers handling microorganisms from exposure by aerosol containing microorganisms generated during work and to prevent leakage to surrounding areas.

(v) Risk mitigation at the time of accepting/dispensing microorganisms

Comply with the relevant laws[2-5] when accepting and dispensing microorganisms. When accepting new microorganisms in an institution, the institution should define a laboratory biosafety level (BSL) by assessing the microbiological risks, and determine in advance necessary items such as measures for emergency and exposure. Before dispensing, check the laboratory biosafety of the recipient. For general matters, refer to the laws, notices, and administrative communications, etc. that describe them in detail.

(vi) Risk mitigation on transporting microorganisms

When transporting microbial samples, take appropriate measures to prevent leakage even when transporting within controlled areas. When transporting outside the controlled areas, it is fundamental to apply triple packaging that prevents the sample from leaking[12]. Comply with the laws[2-5] when transporting outside facilities.

(vii) Risk mitigation of infectious wastes

Inactivate infectious wastes absolutely by disinfectants suitable for target microorganisms or by autoclaving. Inactivation treatment should be completed within a controlled area.

(viii) Risk mitigation in case of emergency

Document appropriate measures in preparation for emergency such as microbial exposure or leakage. The measures should include communication methods, maintenance of a communication network, specific measures, stockpiling of necessary devices/tools, and education/training for them. Establish an organization framework to implement them.

3.3. Mitigation of risks concerning with biosecurity

Mitigation of the risk in biosecurity should include the following[13]:

(i) Access controls for workers handling microorganisms

- ID management
- Registration management of workers handling microorganisms
- locking
- Entry/exit management

(ii) Inventory control of microorganisms

- Storage and acceptance/dispense management of microorganisms

3.4. Education and training for biorisk

To improve the skills of workers handling microorganisms, perform educational training for understanding risks in handling microorganism and seafty measures for them. The characteristics of microorganisms, risks due to work, acquisition and training of good microbiological technique (GMT), emergency response, etc. are important. The education/training should be repeated.

3.5. Compliance with relevant laws and regulations

Regarding the handling of specified microorganisms, etc. in the laws[2-5], comply with the relevant laws regarding possession, management and transport of microorganisms and toxins. For general matters, refer to the laws, notices, and administrative communications, etc. that describe them in detail.

4. Review and update of biorisk management

In order to evaluate that biorisk management is functioning effectively, risk assessment, mitigation and performance are periodically reviewed and the management plan is updated. For example, the Plan-Do-Check-Action (PDCA) cycle is a method of appropriate management.

5. References

1) The Japanese Pharmacopoeia Eighteenth Edition, General information ''Basic Concept of Quality Risk Management <*G0-2-170*>''

2) Law No. 114, 1998, ''Act on the Prevention of Infectious Diseases and Medical Care for Patients with Infecti-

ous Diseases'' (April 1, 1999 enforcement).
3) Law No. 166, 1950, ''Act on Domestic Animal Infectious Disease Control'' (Jun 1, 1951 enforcement).
4) Law No. 151, 1950, ''Plant Protection Act'' (May 4, 1950 enforcement).
5) Law No. 97, 2003, ''Act on the Conservation and Sustainable Use of Biological Diversity through Regulations on the Use of Living Modified Organisms'' (February 19, 2004 enforcement).
6) CEN (European Committee for Standardization). CWA (CEN Workshop Agreement) 15793'' Laboratory biorisk management'', Sep. 2011.
7) ISO/DIS 35001: 2019, Biorisk management for laboratories and other related organisations.
8) CEN (European Committee for Standardization). CWA (CEN Workshop Agreement) 16393 ''Laboratory biorisk management-Guideline for the implementation of CWA 15793: 2008'', Jan. 2012.
9) WHO, Laboratory biosafety manual Third Edition, 2004. ISBN 92-4-154650-6.
10) February 1, 1961, Ministry of Health, Labour and Welfare Ordinance No. 2 ''Regulations for Buildings and Facilities for Pharmacies'', Article 8 ''Buildings and Facilities of Manufacturing Sites for Manufacturers of Specified Biological Drugs''
11) December 24, 2004, Ministry of Health, Labour and Welfare Ordinance No. 179 ''Ministerial Ordinance on Standards for Manufacturing Control and Quality Control of Pharmaceuticals and Quasi-drugs'' Chapter 2 Section 4 ''Manufacturing Management and Quality Control of Biological Drugs''.
12) WHO, Guidance on regulations for the Transport of Infectious Substances 2013-2014.
13) WHO, Biorisk management: Laboratory biosecurity guidance, 2006.

Table 1 Classification of microbiological risk level

Microbiological risk level	criteria
1	There is no or low risk for workers handling microorganisms and related persons. A microorganism that is unlikely to cause human and animal disease (those that do not cause disease to healthy humans).
2	Moderate risk for workers handling microorganisms and low risk for related persons. It can cause disease if it infects humans or animals, but it is unlikely to cause serious health damage to workers handling microorganisms and related persons. There are effective treatments and preventive measures, and low risk of spread to related persons. Many people already have acquired immunity to it and the infection can be easily prevented.
3	High risk for workers handling microorganisms, low risk for related persons. Infection with humans or animals causes serious disease, but is usually less likely to be transmitted from infected persons to related persons. There are effective treatments and preventive measures.
4	High risk to workers handling microorganisms and related persons. Infecting humans or animals can cause serious disease, and transmission from infected persons to related persons can occur directly or indirectly. Usually there is no effective treatment or preventive measures.

Table 2 Classification of laboratory biosafety Level (BSL) and measures

BSL classification	Safety management	Personal protective equipment	Safety equipment	Facility/ equipment (physical containment level)
BSL1	GMT and management system (management organization, handling procedures, education/ training)	Personal protective equipment	Safety equipment	P1 (Basic laboratory)
BSL2	Standard microbial handling procedures corresponding to the microbiological risk level 2, in addition to the requirements of BSL1	In addition to the requirements of BSL1, personal protective equipment complying with the microbiological risk level 2	Safety equipment complying with the microbiological risk level 2, in addition to the requirements of BSL1	P2 (Basic laboratory complying with the microbiological risk level 2)
BSL3	Dedicated standard microbial handling procedures corresponding to the microbiological risk level 3, in addition to BSL2 requirements	In addition to the requirements of BSL2, dedicated personal protective equipment complying with the microbiological risk level 3	Dedicated safety equipment complying with the microbiological risk level 3, In addition to BSL2 requirements	P3 (physical containment laboratory)
BSL4	Dedicated standard microbial handling procedures corresponding to the microbiological risk level 4, in addition to the requirements of BSL3	In addition to the requirements of BSL3, dedicated personal protective equipment complying with the microbiological risk level 4	Dedicated safety equipment complying with the microbiological risk level 4, in addition to the requirements of BSL3	P4 (high grade physical containment laboratory)

Comprehensive risk management methods are classified from BSL1 to BSL4 according to each microbiological risk level, and measures are added and strengthened in order according to newly generated and concerned risks as the BSL value increases. In particular, BSL3 and BSL4 require the use of dedicated good microbiological technique, personal protective equipment and safety equipment.

G5 Crude Drugs

On the Scientific Names of Crude Drugs listed in the JP <*G5-1-181*>

Change the following as follows:

Scientific Names used in the JP and Those being used Taxonomically

Crude Drug	Scientific names used in the JP = Scientific names being used taxonomically (Combined notation, Standard form for author or authors)	Family
	Scientific names that are different from those written in JP but identical to them taxonomically or being regarded as identical, and typical sub-classified groups belonging to their species. The names marked with ''*'' are those being written together in JP.	
Akebia Stem モクツウ	*Akebia quinata* Decaisne = *Akebia quinata* (Thunb. ex Houtt.) Decne.	*Lardizabalaceae*
	Akebia trifoliata Koidzumi = *Akebia trifoliata* (Thunb.) Koidz.	
	Interspecific hybrid between above species	
Clove チョウジ Clove Oil チョウジ油	*Syzygium aromaticum* Merrill et L. M. Perry = *Syzygium aromaticum* (L.) Merr. & L. M. Perry	*Myrtaceae*
	* *Eugenia caryophyllata* Thunberg = *Eugenia caryophyllata* Thunb. *Eugenia caryophyllus* (Spreng.) Bullock & S. G. Harrison	
Gardenia Fruit サンシシ	*Gardenia jasminoides* J. Ellis	*Rubiaceae*
	Gardenia jasminoides J. Ellis f. *longicarpa* Z. W. Xie & M. Okada	
Glehnia Root and Rhizome ハマボウフウ	*Glehnia littoralis* F. Schmidt ex Miquel = *Glehnia littoralis* F. Schmidt ex Miq.	*Umbelliferae*
Magnolia Bark コウボク	*Magnolia obovata* Thunberg = *Magnolia obovata* Thunb.	*Magnoliaceae*
	* *Magnolia hypoleuca* Siebold et Zuccarini = *Magnolia hypoleuca* Siebold & Zucc.	
	Magnolia officinalis Rehder et E. H. Wilson	
	Magnolia officinalis Rehder et E. H. Wilson var. *biloba* Rehder et E. H. Wilson	
Sinomenium Stem and Rhizome ボウイ	*Sinomenium acutum* Rehder et E. H. Wilson = *Sinomenium acutum* (Thunb.) Rehder & E. H. Wilson	*Menispermaceae*

G6 Drug Formulation

Tablet Friability Test <*G6-5-181*>

Change the following as follows:

This test is harmonized with the European Pharmacopoeia and the U.S. Pharmacopeia.

Information on the harmonization with the European Pharmacopoeia and the U.S. Pharmacopeia is available on the website of the Pharmaceuticals and Medical Devices Agency.

The Tablet Friability Test is a method to determine the friability of compressed uncoated tablets. The test procedure presented in this chapter is generally applicable to most compressed tablets. Measurement of tablets friability supplements other physical strength test, such as tablet crushing strength.

Apparatus

Use a drum, with an internal diameter between 283.0 and 291.0 mm and a depth between 36.0 and 40.0 mm, of transparent synthetic polymer with polished internal surfaces, and subject to minimum static build-up (see Fig. 1 for a typical apparatus). One side of the drum is removable. The tablets are tumbled at each turn of the drum by a curved projection with an inside radius between 75.5 and 85.5 mm that extends from the middle of the drum to the outer wall. The outer diameter of the central ring is between 24.5 and 25.5 mm. The drum is attached to the horizontal axis of a device that rotates between 24 and 26 rpm. Thus, at each turn the tablets roll or slide and fall onto the drum wall or onto each other.

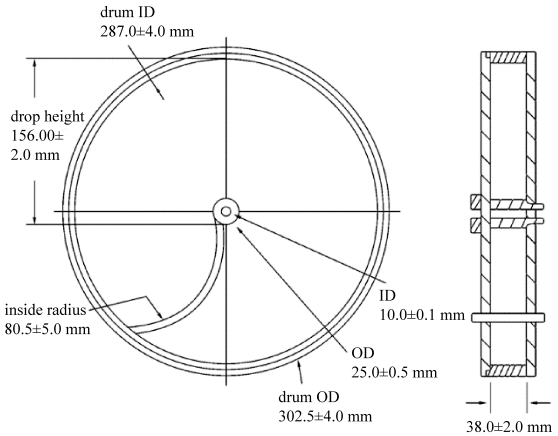


Fig. 1 Tablet friability apparatus

Procedure

For tablets with a unit mass equal to or less than 650 mg, take a sample of *n* whole tablets corresponding as near as possible to 6.5 g. For tablets with a unit mass of more than 650 mg, take a sample of 10 whole tablets. The tablets should be carefully dedusted prior to testing. Accurately weigh the tablet sample, and place the tablets in the drum. Rotate the drum 100 times, between 24 and 26 rpm and remove the tablets. Remove any loose dust from the tablets as before, and accurately weigh.

Generally, the test is run once. If obviously cracked, cleaved, or broken tablets are present in the tablet sample after tumbling, the sample fails the test. If the results are difficult to interpret or if the mass loss is greater than the targeted value, the test should be repeated twice and the mean of the three tests determined. A mass loss from a single test or a mean mass loss from the three tests of not more than 1.0% is considered acceptable for most products. Typically, in case of effervescent and chewable tablets the friability specifications may be different.

If tablet size or shape causes irregular tumbling, adjust the drum base so that the base forms an angle of about 10° with the horizontal and the tablets no longer bind together when lying next to each other, which prevents them from falling freely.

In the case of hygroscopic tablets, an appropriate humidity environment is required for testing.

Drums with dual scooping projections, or apparatus with more than one drum, designed for the running of multiple samples at the same time, are also permitted.

G9 Pharmaceutical Excipients

Add the following:

Functionality-Related Characteristics of Excipients Relevant to Preparations <*G9-1-181*>

Functionality-Related Characteristics (FRC) of excipients are the physical and chemical properties of excipients which are closely relevant to the improvement of the usefulness of active pharmaceutical ingredients and preparations in the manufacturing process, storage, and use of the preparations.

As described in the General Rules for Preparations [1] General Notices for Preparations (6), excipients ''must be pharmacologically inactive and harmless in the administered amount'', and play roles ''to increase the utility of the active substance(s) and preparation, to make formulation process easier, to keep the product quality, to improve the usability, and so forth.'' In the monographs of excipients, specifications and test methods are prescribed for the main purpose of identifying substances and ensuring qualities.

FRC may be effective parameters for excipients to play the above roles, however, no criteria are set for the test methods since the FRC required for excipients depend on the purpose of the use and the formula of preparations and

are different from the quality characteristics that are directly related to the safety and stability of excipients. In addition, the test methods of FRC described in this section do not limit the application of other appropriate test methods.

The FRC of Yellow Petrolatum and White Petrolatum, and the recommended test method for reference are shown below.

Yellow Petrolatum, White Petrolatum: Test method for consistency

Yellow Petrolatum and White Petrolatum are purified mixtures of hydrocarbons obtained from petroleum, and are generally used as the base of semi-solid preparations such as ointments. Ointments are defined in the [3] General Rules for Preparations 11.4. Ointments (3) as to ''have a suitable viscosity for application to the skin'', and their hardness/softness, one of the rheological properties of the dosage form, can be shown by measuring the consistency as a characteristic parameter. The test method to determine the consistency of Yellow Petrolatum and White Petrolatum according to Method 2 under Rheological Measurements for Semi-solid Preparations <*6.16*> is as follows.

(i) Apparatus Perform the test using a standard cone or an optional cone. The containers for the test are flat-bottom metal cylinders that are 100 ± 6 mm in diameter and not less than 65 mm in height.

(ii) Procedure Place the required number of empty containers in an oven, and bring them and a quantity of test substance in a container with a cover to a temperature of 82 ± 2.5°C, pour the Yellow Petrolatum or White Petrolatum into one or more of the containers, filling to within 6 mm of the rim. Cool to 25 ± 2.5°C over a period of not less than 16 hours, protected from drafts. Two hours before the test, place the containers in a water bath at 25 ± 0.5°C. If the room temperature is below 23.5°C or above 26.5°C, adjust the temperature of the cone to 25 ± 0.5°C by placing it in the water bath. Without disturbing the surface of the substance under test, place the container on the penetrometer table, and lower the cone until the tip just touches the top surface of the test substance at a spot 25 mm to 38 mm from the edge of the container. Adjust the zero setting and quickly release the plunger, then hold it free for 5 seconds. Secure the plunger, and read the total penetration from the scale. Make three or more trials, each so spaced that there is no overlapping of the areas of penetration. Where the penetration exceeds 20 mm, use a separated container of the test substance for each trial. Read the penetration to the nearest 0.1 mm. Calculate the average of the three or more readings.

GZ Others

Quality Control of Water for Pharmaceutical Use <*GZ-2-181*>

Change 4.5 Physicochemical Monitoring and below as follows:

4.5. Physicochemical Monitoring

Physicochemical monitoring of a pharmaceutical water processing system is usually performed using conductivity and TOC as the indicators. By monitoring conductivity, total amounts of inorganic salts present in the water can be estimated, and by monitoring TOC, total amount of organic compounds present in the water can be estimated. Normally, Conductivity Measurements <*2.51*> and Test for Total Organic Carbon <*2.59*> specified in the General Tests, Processes and Apparatus of the JP should be applied to these physicochemical monitoring. However, since tests for monitoring are performed in the situations different from those for judging pass/fail to the acceptance criteria prescribed in the monographs, supplements necessary to cover the situations to which the JP general tests cannot be applied, are described below.

To adopt the monitoring using conductivity and TOC as the indicators for inorganic and organic impurities at individual facility, appropriate alert and action levels for each indicator, and countermeasures against unexpected apparatus failures should be established.

4.5.1. Monitoring of Conductivity

Measurement of conductivity for monitoring is usually conducted continuously using an in-line apparatus with a flow-through type or pipe-insertion type cell. Alternatively, offline batch testing may be performed using a dip type cell with water specimens taken at appropriate locations of the pharmaceutical water processing system.

(1) On-line or in-line measurement

Usually, it is somewhat difficult to control the temperature exactly in in-line conductivity monitoring. Therefore, the following approach can be applied for the monitoring at any temperature.

(i) Determine the temperature and the conductivity of the water specimens using a non-temperature-compensated conductivity reading.

(ii) From the Table 3, find the temperature value equal to or just lower than the measured temperature. Adopt the corresponding conductivity value on this table as the allowable conductivity at the measured temperature.

(iii) If the observed conductivity is not greater than the allowable conductivity adopted above, the water tested meets the requirement for monitoring conductivity. If the observed conductivity exceeds the allowable conductivity, off-line measurement is performed.

Table 3 Allowable Conductivity for Different Temperatures*

Temperature (°C)	Allowable Conductivity ($\mu S \cdot cm^{-1}$)	Temperature (°C)	Allowable Conductivity ($\mu S \cdot cm^{-1}$)
0	0.6		
5	0.8	55	2.1
10	0.9	60	2.2
15	1.0	65	2.4
20	1.1	70	2.5
25	1.3	75	2.7
30	1.4	80	2.7
35	1.5	85	2.7
40	1.7	90	2.7
45	1.8	95	2.9
50	1.9	100	3.1

* Applicable only to non-temperature-compensated conductivity measurements.

(2) Off-line Measurement

(i) Measure the conductivity of the water specimen, by transferring it into a container and agitating it vigorously in order to attain equilibrium between the water specimen and the atmosphere on absorbing/desorbing carbon dioxide.

(ii) Transfer a sufficient amount of water to be tested into a suitable container, and stir the water specimen. Adjust the temperature to 25 ± 1°C, and begin agitating the water specimen vigorously, while observing the conductivity periodically. When the change in conductivity becomes not greater than 0.1 $\mu S \cdot cm^{-1}$ per 5 minutes, adopt the observed value as the conductivity (25°C) of the water specimen.

(iii) If the conductivity of the water specimen at 25°C obtained above is not greater than 2.1 $\mu S \cdot cm^{-1}$, the water tested meets the requirement for monitoring conductivity. If the observed value exceeds 2.1 $\mu S \cdot cm^{-1}$, it should be judged that the water tested does not meet the requirement for monitoring conductivity.

4.5.2. TOC Monitoring

The acceptance criterion of TOC is specified as ''not greater than 0.50 mg/L (500 ppb)'' in the monographs of *Purified Water* and *Water for Injection*. However it is recommended for each facility preparing pharmaceutical water to conduct the operation control of a pharmaceutical water processing system through TOC monitoring on produced water based on its own alert and action levels for TOC determined individually. The following are the recommended action levels for TOC.

• Action Level: ≦ 300 ppb (in-line)
≦ 400 ppb (off-line)

The Quality Standards for Drinking Water provided under the Article 4 of the Japanese Water Supply Law require that TOC of tap water (*Water*) should be ''not greater than 3 mg/L (3 ppm)''. Taking the above recommended action levels into consideration, it is also recommended for each facility to conduct quality control of source water through TOC monitoring based on its own alert and action levels for TOC determined individually.

The JP specifies Test for Total Organic Carbon <*2.59*>, and normally, TOC measurement should be conducted using an apparatus which meets the requirements described in the JP method. However, if a TOC apparatus conforms to the apparatus suitability test requirements described in ''< 643> TOTAL ORGANIC CARBON'' of the USP, or those described in the ''*Methods of Analysis 2.2.44*. TOTAL ORGANIC CARBON IN WATER FOR PHARMACEUTICAL USE'' of the EP, the apparatus can be used for the monitoring of a pharmaceutical water processing system, if sufficiently pure water not contaminated with ionic organic substances, or organic substances having nitrogen, sulfur, phosphorus or halogen atoms in their chemical structures, is used as the source water supplied to the system.

A TOC apparatus, characterized by calculating the amount of TOC from the difference in conductivity before and after the decomposition of organic substances without separating carbon dioxide from the sample solution, may be influenced negatively or positively, when applied to the water specimens containing ionic organic substances, or organic substances having nitrogen, sulfur, phosphorus or halogen atoms in their chemical structures. Therefore, the apparatus used for TOC monitoring should be selected appropriately in consideration of the purity of the water to be monitored and the contamination risk in the case of apparatus failure.

4.6. Temporary Storage of *Water for Injection*

In storing *Water for Injection* temporarily, adequate measures able to prevent microbial proliferation stringently, such as circulating it in a loop at a high temperature must be taken, and an appropriate storage time should also be established based on the validation studies, in consideration of the risks of contamination and quality deterioration.

5. Points to Consider for Assuring the Quality of Pharmaceutical Water in Containers

There are some specific points to consider for assuring the quality of pharmaceutical water in containers (*Purified Water in Containers*, *Sterile Purified Water in Containers* and *Sterile Water for Injection in Containers*), which are available as commercially products.

5.1. Methods for Preparing Sterile Pharmaceutical Water in Containers

The following two different preparation methods are described in the monographs of *Sterile Purified Water in Containers* and *Sterile Water for Injection in Containers*.

(i) Introduce *Purified Water* or *Water for Injection* into a hermetic container, seal up the container, then sterilize the product.

(ii) Make *Purified Water* or *Water for Injection* sterile by using a suitable method, introduce the sterilized water into a sterile hermetic container by applying aseptic manipulation, then seal up the container.

For assuring the sterility of pharmaceutical water

products, only the validation of final sterilization process is required in the case of preparation method (i), whereas validations of all the processes are indispensable in the case of preparation method (ii), since the latter is based on the idea to assure the sterility of pharmaceutical water products by ''aseptically'' introducing *Purified Water* (or *Water for Injection*) treated in advance with filtration sterilization, etc. into a sterile hermetic container, and sealing it up.

5.2. Deterioration of Water Quality during the Storage in Containers

5.2.1. Inorganic impurities (Conductivity as the indicator)

The conductivity of pharmaceutical water in containers may increase to some higher levels due to the absorption of carbon dioxide from the atmosphere at the time of its preparation and that passed through plastic layer of the containers during storage, and also due to ionic substances released from the containers, even if the conductivity of *Purified Water* or *Water for Injection* used for its production is maintained at the level not more than $1.3\,\mu S \cdot cm^{-1}$ (25°C). Particularly in the cases of pharmaceutical water products packed in small scale glass containers, it is necessary to pay attention to the change of conductivity during storage.

5.2.2. Organic impurities (Potassium Permanganate-reducing Substances or TOC as the indicator)

The JP specifies the classical test of potassium permanganate-reducing substances in the monographs of *Purified Water in Containers*, *Sterile Purified Water in Containers* and *Sterile Water for Injection in Containers* for controlling organic impurities in pharmaceutical water in containers. It forms a remarkable contrast to the specifications of *Purified Water* and *Water for Injection*, in which the JP requires to control organic impurities in pharmaceutical water in bulk based on the test of TOC (acceptance criterion: not more than 0.5 mg/L (500 ppb)). This is because that it is considered difficult to establish the specification of pharmaceutical water in containers for organic impurities based on the test of TOC from the facts that there were many cases of remarkable increases in TOC values after storage of water in containers. Particularly in the cases of pharmaceutical water products packed in small scale plastic containers, it is necessary to pay attention to the increase of materials released from containers during storage.

The test of potassium permanganate-reducing substances is retained in the specifications of pharmaceutical water in containers, not as the most suitable method for the test of organic impurities present in the water in containers, but as a counter measure for performing the test of the water in containers with the same test method despite of the material (glass, polyethylene, polypropylene, etc.) and the size (0.5 – 2000 mL) of the containers, and the duration of storage. Therefore, it is recommended to adopt the test of TOC as the alternative for the test of potassium permanganate-reducing substances, and to perform quality control of pharmaceutical water in containers based on TOC measurements under the responsibility of each manufacturer, if possible. In such cases, it is recommended to adopt the following values as the levels preferable to attain.

For products containing not more than 10 mL of water:
TOC not greater than 1500 ppb

For products containing more than 10 mL of water:
TOC not greater than 1000 ppb

As for the pharmaceutical water packed in the plastic containers made of polyethylene, polypropylene, etc., in addition to the concern for the release of materials such as monomer, oligomers, plasticizers, etc. from plastics, it is necessary to pay attention to the storage environment of the products to avoid the contaminations with low molecular volatile organics such as alcohol, or low molecular air pollutants such as nitrogen oxides, since these plastics have the properties of permeating various gases and water.

5.2.3. Microbial Limit (Total Aerobic Microbial Count)

For *Purified Water in Containers*, it is not required to assure the sterility, but it is necessary to produce it by using sanitary or aseptic processes in order to meet the acceptance criterion of ''10^2 CFU/mL'' for total aerobic microbial count throughout the period of their storages. It is also necessary to take special care against microbial contamination during its circulation. In addition, it is recommended to use them as soon as possible after opening their seals.

The acceptance criterion of ''10^2 CFU/mL'' for total aerobic microbial count of *Purified Water in Container* is at the same level as the action level for microbial count in the production of *Purified Water* (in bulk). However, different from the case of microbiological monitoring of *Purified Water*, Soybean-Casein Digest Agar Medium is used for the test of total aerobic microbial count of *Purified Water in Containers* to detect microorganisms contaminated from the surroundings during its storage.

5.3. Points to consider in the case that commercially available products of pharmaceutical water in containers are used for the manufacture or the tests of pharmaceutical products

It is allowable to use commercially available products of pharmaceutical water in containers (*Purified Water in Containers*, *Sterile Purified Water in Containers* and *Sterile Water for Injection in Containers*) for the manufacture of pharmaceutical products and products for clinical trial, and for the tests of pharmaceutical products. In such cases, it is necessary to consider the following points.

(i) Use them soon after confirming their compliances to the requirements of the JP monograph from the test results at the time of its receipt or those offered from the supplier of the products.

(ii) In the case that such products are used for manufacturing pharmaceutical products, it is necessary to perform process validation as a part of manufacturing process of pharmaceutical products. In the case that they are used for manufacturing products for clinical trial, it is necessary to confirm that the water doesn't give any adverse effects on the quality of the products.

(iii) The products of sterile pharmaceutical water in containers should be used only once after opening their seals, and it must be avoided to use them again after storage.

(iv) It is recommended to prepare a standard operation

practice (SOP) adequate for its intended use, considering that the contamination and quality deterioration of the water due to human and laboratory environmental origins might go on rapidly immediately after opening the product seal.

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